ISSN: 2158-284X Volume 10, Number 10, October 2019

Scientific Research Publishing

International Journal of Clinical Medicine

ISSN: 2158-284X

https://www.scirp.org/journal/ijcm

Journal Editorial Board

ISSN: 2158-284X (Print) ISSN: 2158-2882 (Online)
https://www.scirp.org/journal/ijcm

International Journal of Clinical Medicine, 2019, 10, 463-575
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Table of Contents

Volume 10 Number 10 October 2019

International Journal of Clinical Medicine (IJCM)
Journal Information

SUBSCRIPTIONS

The *International Journal of Clinical Medicine* (Online at Scientific Research Publishing, https://www.scirp.org/) is published monthly by Scientific Research Publishing, Inc., USA.

Subscription rates:
Print: $79 per issue.
To subscribe, please contact Journals Subscriptions Department, E-mail: sub@scirp.org

SERVICES

Advertisements
Advertisement Sales Department, E-mail: service@scirp.org

Reprints (minimum quantity 100 copies)
Reprints Co-ordinator, Scientific Research Publishing, Inc., USA.
E-mail: sub@scirp.org

COPYRIGHT

PRODUCTION INFORMATION

For manuscripts that have been accepted for publication, please contact:
E-mail: ijcm@scirp.org

International Journal of Clinical Medicine, 2019, 10, 463-468
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Accidental Diagnosis of Infections among Blood Donors

Sara Sadiq[1*], Mahira Lakhani[2], Salman Baig[3], Muhammad Fazal Hussain Qureshi[2], Muzna Shah[2]

[1]Department of Physiology, CMH Institute of Medical Sciences, Bahawalpur, Pakistan
[2]Student 3rd Year MBBS, Ziauddin University, Karachi, Pakistan
[3]Department of Otolaryngyology and Infection Control, Bahria International Hospital, Karachi, Pakistan
Email: dr.sarabhatti@gmail.com

How to cite this paper: Sadiq, S., Lakhani, M., Baig, S., Qureshi, M.F.H. and Shah, M. (2019) Accidental Diagnosis of Infections among Blood Donors. *International Journal of Clinical Medicine*, **10**, 463-468.
https://doi.org/10.4236/ijcm.2019.1010039

Received: September 14, 2019
Accepted: October 11, 2019
Published: October 14, 2019

Abstract

Introduction: Due to widespread incidence and prevalence, human immunodeficiency virus (HIV), hepatitis B (HBV) and C (HCV), syphilis and malaria are the most common viral infections transmitted via blood transfusions. Yet there is insufficient information available about the exact prevalence of these infections among blood donors in Pakistan. The objectives of current study are to highlight the importance of blood screening among blood donors by finding the prevalence of high-risk transmissible diseases such as hepatitis B, hepatitis C, HIV, syphilis and malaria in the population of Nawabshah (Sindh) and to compare it with other provinces. Methods: A descriptive type of cross-sectional study was conducted on 37,845 blood donor volunteers at the blood bank of a tertiary care hospital, Peoples University of Medical and Health Sciences Hospital, in Nawabshah Sindh from 1st January 2018 to 31st December 2018. The data was taken from the well-maintained yearly record of the hospital blood bank. Analysis of all the data regarding blood donors was done using Statistical Program of Special Sciences (SPSS) version 20. Results: The most common age group was between 18 - 40 years. Donors were predominantly male (90%). HBV is the most prevalent disease amongst the blood donors of Peoples Medical College with a prevalence of 6.52% while HIV, HCV and malaria were 6.33%, 5.83% and 1.24% respectively whereas Syphilis is the least prevalent disease with a prevalence of 1.11%. Comparing with other provinces, the highest prevalence of hepatitis B was seen in Peoples Medical College, Nawabshah, Sindh whereas for hepatitis C it was the highest in Isra University Hospital, Hyderabad, Sindh. Moreover, Hayatabad Medical Complex in Peshawar was the least burdened with Hepatitis B (1.46%) and C (1.34%). Conclusion: Accidental diagnosis of blood-borne infections including Hepatitis B, C, HIV, Syphilis and malaria highlights importance of blood screening among the blood donors. There is a need of public education regarding infection prevention and transmission.

Keywords

Blood Donors, Infectious Diseases, Hepatitis B, Hepatitis C

1. Introduction

A vital part of every health care system (Primary, Secondary and tertiary) is blood donation. Blood donation is an indispensable measure for all patients who require transfusion of blood during the disease stage or in emergency situations. Any person who is willing for donating blood without any urge of demand or anything in return is known as a voluntary blood donor. It is a social duty and a great contribution towards community and humanity as it guarantees a continuous supply of blood even if it is as a replacement donor. However, concerned administrative authorities are involved directly in the provision of safe blood supply to control the diseases transmitted by blood transfusion. Due to widespread incidence and prevalence, human immunodeficiency virus (HIV), hepatitis B (HBV) and C (HCV), syphilis and malaria are the most common viral infections transmitted via blood transfusions.

HIV is a virus that leads to many diseases related to immune system; acquired immunodeficiency syndrome (AIDS) has life threatening repercussions. HBV and HCV are amongst the top precipitating factors of liver cirrhosis, carcinoma and end stage liver disease. As stated by the World Health Organization (WHO) about 350 million people have long-standing HBV infection, about 170 million people have persistent HCV infection and 36.9 million people suffer from HIV worldwide [1] [2] [3]. Annually, 663,000, 399,000 and 940,000 deaths occur as a consequence of hepatitis B, hepatitis C and HIV respectively [4].

Pakistan has one of the world's highest fertility rates, with more than four children per woman. On account of its large and ever-increasing population in addition to high rates of infection, Pakistan is significantly burdened with these diseases [1] [2]. Yet there is insufficient information available about the exact prevalence of hepatitis B, hepatitis C, HIV, malaria and syphillus among blood donors in Pakistan. The objectives of current study are to highlight the importance of blood screening among blood donors by finding the prevalence of high-risk transmissible diseases such as hepatitis B, hepatitis C, HIV, syphilis and malaria in the population of Nawabshah (Sindh) and to compare it with other provinces.

2. Methods

A descriptive type of cross-sectional study was conducted on 37,845 blood donor volunteers at the blood bank of a tertiary care hospital, Peoples University of Medical and Health Sciences Hospital, in Nawabshah Sindh. The blood donor's data regarding demographic variables and screening test for HBV, HCV, HIV, syphilis and malaria was collected from 1st January 2018 to 31st December 2018.

The data was taken from the well-maintained yearly record of the hospital blood bank. As per SOPs of hospital all the blood donors were healthy on the record of history and physical examination, for blood donation. Study got approval from the Ethical review committee of Peoples University of Medical and Health Sciences.

Analysis of all the data regarding blood donors was done using Statistical Program of Special Sciences (SPSS) version 20. Frequencies and percentages were used to present the qualitative data. The prevalence of all the screened diseases among donors was calculated while the prevalence of hepatitis B and C was compared with other studies done in different cities of Pakistan.

3. Results

A prospective study was carried out in Peoples Medical College Hospital, Nawabshah from January 2018 to December2018.The total donors were 37,845. Their age was between 18 - 40 years. Donors were predominantly male (90%). HBV is the most prevalent disease amongst the blood donors of Peoples Medical College with a prevalence of 6.52% whereas Syphilis is the least prevalent disease with a prevalence of 1.11% as mentioned in Table 1.

Highest prevalence of Hepatitis B (6.52%) was seen in Peoples Medical College, Nawabshah, Sindh whereas for Hepatitis C it was highest in Isra University Hospital, Hyderabad, Sindh. Moreover, Hayatabad Medical Complex in Peshawar was the least burdened with Hepatitis B (1.46%) and C (1.34%) as mentioned in Table 2.

4. Discussion

In this study, we compared seroprevalence of HBV and HCV in healthy blood donors of People's Medical College, Nawabshah, Sindh with the different hospitals from different provinces. Hepatitis and HIV, similar to other blood borne illnesses, are transmitted sporadically-microepidemics. To curb the transmission of HBV and HCV we must identify the causes of these microepidemics. [18] [19] The variation in seroprevalence amidst comparable regions or populations can be a consequence of methodological differences in sampling strategies.

As reported earlier, Nawabshah appears to be plagued by Hepatitis B to the greatest extent that is 6.52%. If we compare this data with that of Western

Table 1. Prevalence of different diseases for which blood was screened.

	Prevalence (%)	n = 37,845
HIV	6.33	2389
HBV	6.52	2466
HCV	5.83	2208
Syphilis	1.11	421
Malaria	1.24	471

Table 2. Prevalence of Hepatitis B and C among blood donors reported in the last few years in different cities of Pakistan.

Province	City	Place of Research	Time of study	HBsAg (%)	Anti-HCV (%)
Sindh	Karachi [5]	Baqai Medical University	2007	4.5	4.36
	Karachi [6]	Baqai Medical University, PNS Shifa	2008	1.71	2.06
	Hyderabad [7]	Isra University Hospital	2006	3.65	8.68
	Nawabshah (Current Study)	Peoples Medical College	2018	6.52	5.83
Punjab	Rawalpindi [8]	AFIT	2002	3.3	4.0
	Rawalpindi [9]	Islamic International College	2002	5.86	6.21
	Rawalpindi [10]	Fauji Foundation Hospital	2006	2.45	2.52
	Lahore [11]	Shaikh Zayed Postgraduate Medical Institute	2005	3.36	4.16
	Lahore [12]	Ghurki Trust Teaching Hospital	2007	1.52	5.34
	Lahore [13]	Nawaz Sharif Social Security Hospital	2009	1.70	7.69
	Bahawalpur [14]	Quaid-e-Azam Medical College	2006	2.69	2.52
	Islamabad [15]	Shifa International Hospital	2004	2.51	5.14
Balochistan	Quetta [16]	CMH	2003	-	1.87
KPK	Peshawar [17]	Hayatabad Medical Complex	2004	1.46	1.34

Europe and United States of America, we can conclude that the burden of HBV infection is lower there (0.1% - 0.5%) [20]. There are several reasons for this. WHO reports that in Southeast Asia an average person receives nearly four injections per year, mostly unnecessary [21]. Such injections play a crucial role in the spread of HBV and HCV. Other causes include sexual transmission, recipients of infected blood, occupational hazards to health workers and IV drug abusers.

In Sindh, Nawabshah bears majority of the brunt of HBV and HCV infections as opposed to Karachi. In Baqai Medical College, Karachi the prevalence of HBV has dropped from 4.5% to 1.71% and that of HCV from 4.36% to 2.06% over a period of one year (2007-2008). This difference could be because of increased screening of blood, education of sex workers regarding condom use, better disposal of contaminated needles, increased awareness about the risks of unnecessary injections. On the contrary, HBV and HCV infections have a prevalence of 1.46% and 1.34% respectively in KPK which is much less compared to other cities of Pakistan. In KPK, both military personnel and civilians made up the donor population. Life style of those in armed forces and civilians is poles apart; closed community living, barracks, almost uniform working environment and same health facilities. The disparity observed between their seroprevalence rate can be possibly attributed to these reasons [22].

In Pakistan, the seroprevalence of HBV and HCV is high amongst healthy donors of blood. Hence, infected people need to be identified to prevent further transmission and complications. Multiple researchers have identified a few key elements of utmost importance in aiding spread of HBV and HCV; unscreened

blood transfusion, reuse of unsterilized syringes and medical equipment. That being the case, proper disposal of contaminated needles, condom use, apt screening of blood before donation as well as transfusion and vaccination against Hepatitis B should be encouraged. Looking over the other screened infections including HIV, Syphilis and malaria, none of the study has yet reported their prevalence among blood donors in Pakistan. Limitation of the current study is that data should be collected on large scale.

5. Conclusion

Accidental diagnosis of blood-borne infections including Hepatitis B, C, HIV, Syphilis and malaria highlights the importance of blood screening among the blood donors. There is a need for public education regarding infection prevention and transmission.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] WHO (2000) Hepatitis C. World Health Organization, Geneva. https://www.who.int/en/news-room/fact-sheets/detail/hepatitis-c

[2] WHO (2000) Hepatitis B. World Health Organization, Geneva. https://www.who.int/news-room/fact-sheets/detail/hepatitis-b

[3] UNAIDS (2018) Global HIV & AIDS Statistics—2018 Fact Sheet.

[4] Perz, J.F., Armstrong, G.L., Farrington, L.A., Hutin, Y.J. and Bell, B.P. (2006) The Contributions of Hepatitis B Virus and Hepatitis C Virus Infections to Cirrhosis and Primary Liver Cancer Worldwide. *Journal of Hepatology*, **45**, 529-538.

[5] Azam, M., *et al*. (2007) Blood Donor Screening for Hepatitis and HIV. *Journal of Dow University of Health Sciences*, **1**.

[6] Nazar, H., Nadia, N., Shazia, N., Zulfiqar, A. and Farhat, A. (2008) Prevalence of Hepatitis B and Hepatitis C in Blood Donors of Karachi. *Biomedica*, **24**, 116-117.

[7] Ujjan, I., *et al*. (2006) Seroprevalence of HBsAg and Anti-HCV in Healthy Blood Donors. *Pakistan Journal of Gastroenterology*, **20**, 75-77.

[8] Shah, S.M., Khattak, I.U., Ali, A. and Tariq, M. (2010) Seropositivity for Hepatitis B and C in Voluntary Blood Donors. *Journal of Ayub Medical College Abbottabad*, **22**, 149-151.

[9] Mumtaz, S. (2002) Frequency of Seropositive Blood Donors for Hepatitis B, C and HIV Viruses in Railway Hospital Rawalpindi. *PJMR-Pakistan Journal of Medical Research*, **41**, 51-53.

[10] Chaudhary, I.A., *et al*. (2007) Seroprevalence of Hepatitis B and C among the Healthy Blood Donors at Fauji Foundation Hospital, Rawalpindi. *Pakistan Journal of Medical Sciences*, **23**, 64-67.

[11] Rehman, N.A.S., *et al*. (2005) Frequency of Hepatitis B, C and Human Immunodeficiency Virus in Blood Donors at Shaikh Zayed Hospital, Lahore. *Proceeding SZPGMI*, **19**, 33-36.

[12] Ijaz, A., *et al*. (2007) Hepatitis B and Hepatitis C in Blood Donors: Analysis of 2-Years Data. *Annals of King Edward Medical University*, **13**, 59-61.

[13] Anwar, M.S., Mujtaba Siddiqi, G., Haq, S., Ghias Khokhar, N. and Jaffery, G. (2011) Association of Blood Group Types to Hepatitis B and Hepatitis C Virus Infection. *Biomedica*, **27**, 57-61.

[14] Khan, M., *et al*. (2006) Hepatitis B, C & HIV; Seroprevalence of Infection in Blood Donors. *The Professional Medical Journal*, **13**, 632-636.

[15] Asif, N., Khokhar, N. and Ilahi, F. (2004) Sero-Prevalence of HBV, HCV, and HIV Infection among Voluntary Non Remunerated & Replacement Donors in Northern Pakistan. *Pakistan Journal of Medical Sciences*, **20**, 24-28.

[16] Ali, N., Nadeem, M., Qureshi, A.H., Qamar, A. and Ejaz, A. (2003) Frequency of Hepatitis-C Virus Antibodies in Blood Donors in Combined Military Hospital, Quetta. *Pakistan Journal of Medical Sciences*, **19**, 41-44.

[17] Zaidi, A., *et al*. (2008) Seroprevalence of Hepatitis B, C and HIV in Health Blood Donors on Northern Pakistan. *Pakistan Journal of Pathology*, **8**, 100-104.

[18] Centers for Disease Control and Prevention (CDC) (2003) Transmission of hepatitis B and C Viruses in Outpatient Settings-New York, Oklahoma, and Nebraska, 2000-2002. *MMWR*: *Morbidity & Mortality Weekly Report*, **52**, 901-906.

[19] CDC (2005) Transmission of Hepatitis B Virus among Persons Undergoing Blood Glucose Monitoring in Long-Term-Care Facilities—Mississippi, North Carolina, and Los Angeles County, California, 2003-2004. *MMWR*: *Morbidity and Mortality Weekly Report*, **54**, 220.

[20] Busch, M. (2000) HIV, HBV and HCV: New Developments Related to Transfusion Safety. *Vox Sanguinis*, **78**, 253-256.

[21] Janjua, N. (2003) Injection Practices and Sharp Waste Disposal by General Practitioners of Murree, Pakistan. *Journal of Pakistan Medical Association*, **53**, 104-110.

[22] Khattak, M., Salamat, N., Bhatti, F.A. and Qureshi, T.Z. (2002) Seroprevalence of Hepatitis B, C and HIV in Blood Donors in Northern Pakistan. *Journal of Pakistan Medical Association*, **52**, 398-402.

International Journal of Clinical Medicine, 2019, 10, 469-476
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Magnetic Resonance Imaging-Measured Adductor Muscle Volume and 100 m Sprint Running Performance in Female Sprinters

Tomohiro Yasuda[1], Kazuhisa Kawamoto[2], Jeremy P. Loenneke[3], Takashi Abe[3*]

[1]School of Nursing, Seirei Christopher University, Shizuoka, Japan
[2]Faculty of Human Development and Culture, Fukushima University, Fukushima, Japan
[3]Department of Health, Exercise Science and Recreational Management, Kevser Ermin Applied Physiology Laboratory, The University of Mississippi, University, MS, USA
Email: *t12abe@gmail.com

How to cite this paper: Yasuda, T., Kawamoto, K., Loenneke, J.P. and Abe, T. (2019) Magnetic Resonance Imaging-Measured Adductor Muscle Volume and 100 m Sprint Running Performance in Female Sprinters. *International Journal of Clinical Medicine*, **10**, 469-476.
https://doi.org/10.4236/ijcm.2019.1010040

Received: September 18, 2019
Accepted: October 11, 2019
Published: October 14, 2019

Abstract

The purpose of this study was to determine the specific muscles that may contribute to sprint performance. Eleven female 100-m sprinters and nine non-sprinters volunteered. Thigh muscle volume (MV) was measured using magnetic resonance imaging (MRI) images obtained from the spina iliaca anterior-superior to below the distal end of the femur. The MV of the adductors, quadriceps and hamstrings was calculated. Evidence for the null/alternative hypothesis was provided thorough the calculation of Bayes Factors (BF_{10}). Differences represented as median δ (95% credible interval). Absolute MVs in the quadriceps [1.287 (0.315, 2.39), BF_{10}: 14.3], hamstrings [3.032 (1.886, 4.482), BF_{10}: 9487.4] and adductors [3.22 (1.994, 4.654), BF_{10}: 23,360.2] were greater in sprinters than in non-sprinters. This was also observed when MV was normalized to body mass (cm^3/kg). Absolute and relative MVs in the adductor longus, the adductor brevis, the adductor magnus, pectineus, and gracilis were also greater in the sprinters. However, percentage of component adductor relative to total adductors MV appeared similar between the two groups. There was no evidence for a correlation between sprint time and quadriceps, hamstrings and adductors MV relative to body mass. Within the adductors, there was evidence for a correlation between sprint time and adductor brevis MV relative to body mass [r = −0.652, BF_{10}: 3.028, δ −0.548 (−0.870, 0.040)]. Although the credible interval was wide, our results suggest that the adductor brevis may contribute to sprint running performance in female sprinters. This may be related, in part, to supporting the flexion and medial rotation of the thigh.

Keywords

Body Composition, Female Athletes, Magnetic Resonance Imaging, Muscle Mass, 100-m Dash

1. Introduction

Magnetic resonance imaging (MRI) of the lower extremities and trunk muscles in sprinters has been studied by several researchers [1] [2] [3] [4] in an effort to examine the specific muscles that contribute to sprint running performance (*i.e.* best 30 - 100 m dash time or maximal running speed). For instance, Hoshikawa *et al.* [2] reported that a larger muscle cross-sectional area (CSA) of the psoas major relative to quadriceps muscle CSA was correlated with a season-best 100 m time in 22 junior male and 22 junior female sprinters. Similarly, Copaver *et al.* [1] observed a correlation between sprint performance (50 m and 120 m dash times) and psoas major muscle CSA as well as hip flexion power in 10 Afro Caribbean athletes including soccer, tennis, track and field, and combat sports. Recently, a study by Sugisaki *et al.* [4] reported correlations between the season best 100 m time and absolute muscle volume of the psoas major, gluteus maximus, gluteus medius plus minimus, and hamstrings, while other muscles include adductors which did not correlate to the performance in 31 Japanese male sprinters. These findings suggest that the psoas major and gluteus maximus, which act as flexion or extension of the thigh, may be specific muscles that contribute to sprint running performance.

The adductors consist of three major muscles namely the adductor longus, adductor brevis, and adductor magnus with the primary function known as a hip adduction and a medially/laterally rotation of the thigh [5]. During sprint running, however, these muscles perform different functions at different moments of the action. For example, the adductor longus located in anteromedial thigh acts to flex the hip just after toe-off and remains active during the follow-through and early forward swing [6], while the adductor magnus located in posteromedial thigh acts to extend the hip from the flexed position during backward swing [7]. From the studies of electromyography [6] [7], the functions of the adductors may be not only hip adduction and lateral rotation of the thigh but also contributes to hip extension and flexion during sprint running. However, it is unknown how each individual adductor correlates with sprint performance in sprinters. Thus, the purpose of this study was to determine how muscle volume differed between sprinters and non-sprinters and determine the relationship between individual adductor muscles and sprint performance. If a correlation does exist, this might suggest an important role of that particular muscle during sprinting and may offer a target of emphasis for future researchers and practitioners.

2. Methods

Eleven female competitive 100-m sprinters (competing at least at national level) and nine age-matched college students (non-sprinters) were recruited for the study. The body composition of the sprinters and non-sprinters are indicated in Table 1. The best official time (100 m) was used for evaluating sprint performance. The sprinters contain five elite sprinters who participated in the World Championships and/or Asian Games and the other six sprinters were members of the university's track and field team. All sprinters were involved in regular sprint training at least five times a week and resistance training two times per week. The non-sprinters were healthy university students and were physically active performing aerobic-type exercise (*i.e.* jogging and swimming) regularly 2 - 3 times a week (approximately 30 min each session), but none of the participants had participated in regular strength/resistance training for a minimum of 3 years prior to the start of the study. The study was conducted according to the Declaration of Helsinki and was approved by the university's Ethics Committee for Human Experiments. Written informed consent was obtained from all the participants.

Subcutaneous fat thickness was measured using ultrasound as described previously [8]. Body density was estimated from subcutaneous fat thickness using

Table 1. Body composition, thigh muscle volume and 100 m sprint performance in sprinters and non-sprinters.

	Non-Sprinter	Sprinter	Median δ (95% credible interval)	BF_{10}
Age, years	21.1 (2.2)	22.5 (3.8)	−0.259 (−1.068, 0.463)	0.516
Standing height, m	1.58 (0.05)	1.63 (0.05)	−0.667 (−1.598, 0.146)	1.599
Body mass, kg	52.0 (6.4)	53.5 (3.7)	−0.175 (−0.971, 0.525)	0.456
Body mass index, kg/m^2	21.0 (3.1)	20.2 (1.1)	0.221 (−0.467, 1.005)	0.492
Body fat, %	22.6 (4.4)	12.5 (0.7)	3.081 (1.810, 4.502)	15,739.5
Fat-free mass, kg	40.2 (5.2)	46.8 (3.3)	−1.255 (−2.313, −0.300)	13.2
Muscle volume, cm^3				
Quadriceps	1082 (237)	1418 (193)	−1.287 (−2.390, −0.315)	14.3
Hamstrings	381 (94)	648 (73)	−3.032 (−4.482, −1.886)	9487.4
Adductors	567 (115)	907 (82)	−3.22 (−4.654, −1.994)	23,360.2
Muscle volume relative to body mass, cm^3/kg				
Quadriceps	20.7 (2.9)	26.4 (2.1)	−2.040 (−3.280, −0.957)	274.7
Hamstrings	7.2 (1.2)	12.1 (1.0)	−4.350 (−6.014, −3.129)	1.030e+06
Adductors	10.8 (1.2)	16.9 (0.8)	−5.312 (−7.254, −4.192)	4.890e+07
100m sprint time, s		12.04 (0.5)	-	-

BF_{10}: Bayes Factor ≥ 3 is suggestive of evidence for the alternative hypothesis whereas a Bayes factor of ≤0.33 is suggestive of evidence for the null hypothesis.

an ultrasound-derived prediction equation [9]. Percent body fat was calculated from body density using Brozek and colleague's equation [10]. Fat-free mass was estimated as total body mass minus fat mass. Body mass and standing height were measured to the nearest 0.1 kg and 0.1 cm, respectively, by using an electronic weight scale and a height scale. Body mass index was calculated as body mass/standing height squared (kg/m^2).

Series cross-sectional images of the thigh were obtained by magnetic resonance imaging (MRI) scans with a body coil (Signa 1.5T, GE, Milwaukee, Wisconsin, USA) as described previously [11] [12]. Briefly, the subjects lay supine in the body coil with the hip and knee being the full extension. Transverse scans were carried out every 10-mm from the spina iliaca anterior-superior to 10-cm below the distal end of the femur. Spin-echo, axial-plane imaging of the right-thigh was performed with 1500-ms repetition time, 16.7-ms effective echo time, 1 excitation, 384 × 256 matrix, 25-cm field of view, 10-mm slice thickness and 0-mm interslice gap. For each axial scan, muscle CSA computation was carried out on the quadriceps, hamstrings, and adductors (*i.e.* adductor longus, adductor brevis, adductor magnus, pectineus, and gracilis). From each cross-sectional image, outlines of each muscle were traced, and digitized by using a personal computer, and the muscle CSA was calculated. By summing the total CSA of the muscle along thigh length and then multiplying the sum by the interval of 10-mm, absolute muscle volume was determined. The estimated coefficient of variation of this MV measurement from test-retest (n = 5) was 2% [11].

A Bayesian independent samples t-test was used to determine if there were group differences in body composition and absolute and relative muscle volume of each thigh muscle with a default prior of 0.707. A Bayes factor (BF_{10}) of ≥3 and ≤0.33 was considered evidence for the alternative and null hypotheses, respectively. For the female sprinters, we performed a correlation between sprint time and muscle volume relative to body mass using a default stretched beta prior width of 1. All Bayes factors were calculated using JASP version 0.9.0.1. Data is presented as mean (standard deviation) unless otherwise stated.

3. Results

There were no differences in age, height, body mass and body mass index between sprinters and controls. However, sprinters had lower percent fat and higher fat-free mass compared to non-sprinters (**Table 1**). Absolute muscle volumes and muscle volume relative to body mass in the quadriceps, hamstrings and adductors were greater in sprinters than in non-sprinters (**Table 1**). Similar results were observed in absolute and relative muscle volume in the adductor longus, the adductor brevis, the adductor magnus, pectineus, and gracilis between sprinters and non-sprinters. However, the percentage of each individual muscle to the adductor muscle volume appeared similar between the two groups (**Table 2**).

In sprinters, percent body fat did not correlate with 100 m sprint time. There was no evidence for a correlation between sprint time and quadriceps muscle

Table 2. Absolute and relative muscle volume of individual adductor muscles.

	Non-Sprinter	Sprinter	Median δ (95% credible interval)	BF_{10}
Muscle volume (cm^3)				
Adductor longus	88 (25)	144 (24)	−2.057 (−3.241, −0.590)	297.9
Adductor brevis	69 (16)	113 (21)	−2.041 (−3.271, −0.873)	274.5
Adductor magnus	332 (59)	524 (52)	−3.223, (−4.687, −2.00)	25,056.4
Pectineus	28 (6.7)	39 (7)	−1.292 (−2.390, −0.337)	14.8
Gracilis	51 (23)	87 (11)	−1.775 (−2.923, −0.712)	91.2
Muscle volume relative to body mass, cm^3/kg				
Adductor longus	1.67 (0.34)	2.68 (0.34)	−2.744 (−4.063, −1.540)	4147.9
Adductor brevis	1.31 (0.22)	2.10 (0.31)	−2.604 (−3.925, −1.053)	2526.5
Adductor magnus	6.37 (0.72)	9.81 (0.84)	−4.066 (−5.797, −2.710)	466,876.1
Pectineus	0.54 (0.10)	0.73 (0.12)	−1.411 (−2.485, −0.421)	23.4
Gracilis	0.95 (0.31)	1.62 (0.17)	−2.484 (−3.893, −1.396)	1442.0
Percentage of total adductors muscle volume, %				
Adductor longus	15.3 (2.4)	15.9 (2.2)	−0.144 (−0.920, 0.567)	0.439
Adductor brevis	12.1 (1.3)	12.4 (1.7)	−0.108 (−0.870, 0.604)	0.420
Adductor magnus	58.9 (4.0)	57.9 (3.4)	0.174 (−0.523, 0.963)	0.453
Pectineus	4.9 (0.9)	4.3 (0.7)	0.566 (−0.200, 1.494)	1.167
Gracilis	8.7 (2.3)	9.5 (0.9)	−0.333 (−1.161, 0.365)	0.614

BF_{10}: Bayes Factor ≥ 3 is suggestive of evidence for the alternative hypothesis whereas a Bayes factor of ≤0.33 is suggestive of evidence for the null hypothesis.

volume relative to body mass [r = −0.186, BF_{10}: 0.423, median δ (95% credible interval) −0.145 (−0.640, 0.409)], hamstring muscle volume relative to body mass [r = −0.416, BF_{10}: 0.765, median δ (95% credible interval) -0.332 (−0.760, 0.223)], or adductor muscle volume relative to body mass [r = −0.447, BF_{10}: 0.866, median δ (95% credible interval) −0.358 (−0.775, 0.194)]. Within the adductors, there was no evidence for a correlation between sprint time and adductor longus muscle volume relative to body mass [r = 0.162, BF_{10}: 0.409, median δ (95% credible interval) 0.126 (−0.426, 0.627)] or adductor magnus muscle volume relative to body mass [r = −0.259, BF_{10}: 0.482, median δ (95% credible interval) −0.203 (−0.680, 0.355)]. However, there was evidence for a correlation between sprint time and adductor brevis muscle volume relative to body mass [r = −0.652, BF_{10}: 3.028, median δ (95% credible interval) −0.548 (−0.870, 0.040)].

4. Discussion

The main findings of the current study were that 1) adductors muscle volume was approximately 60% greater in female 100 m sprinters compared to age- and height-matched non-sprinters; 2) the percentage of each individual adductor

muscle relative to the total adductor muscle volume appeared similar between the two groups; and 3) there was a significant correlation between adductor brevis muscle volume relative to body mass and 100 m sprint time in female sprinters. Of note, the total adductor muscle volume as well as other individual muscles within the adductors did not correlate with sprint performance.

There are few studies investigating the individual muscles making up the total thigh volume as measured by MRI in athletes. In the present study, total adductor muscle volume of female sprinters was 907 cm^3 on average. This absolute muscle volume of the adductors was similar as the value of physically active young students (913 cm^3) reported by Ogawa *et al.* [13] Recently, Sugisaki *et al.* [4] reported MRI-measured muscle volume of the adductor splus gracilisin male 100 m sprinters (1278 cm^3). The difference in adductors muscle volume between male and female sprinters was approximately 370 cm^3, while the difference in the quadriceps muscle volume was approximately 800 cm^3 (2210 cm^3 in male sprinters and 1418 cm^3 in female sprinters). Calculated percentage of adductors relative to total thigh muscle volume was relatively higher in our female sprinters (31%) compared to male sprinters (28%). However, the percentage value of our female non-sprinters was 28%. The significance of this potential sex difference is not known and future research is needed to better understand how the adductors might change with sprint training.

In the present study, we found that adductor brevis muscle volume relative to body mass was correlated with 100 m sprint time. Our data suggests that we are 95% confident that the true effect lies between an r value of −0.87 and an r value of 0.040. Although the credible interval was wide, this particular muscle may suggest a target of investigation in future studies. This is the first study to investigate the correlation between the muscle volume of each individual muscle within the adductors and sprint performance in sprinters. Previous studies reported that there were no correlations between total adductors muscle volume or adductor muscle CSA relative to body mass and sprint performance [3] [4]. In line with the previous studies, our results also indicated no correlation between total adductor muscle volume and 100 m sprint time in female sprinters. Previously reported electromyographic studies [6] [7] measured muscle activity (fine wire electrodes system) of the lower body muscles. Although the adductor longus and adductor magnus were examined during sprint running, the adductor brevis was not measured. This may be related, in part, to the difficulty of measuring muscle activity in this muscle (location is inguinal area and relatively deep). The adductor brevis muscle acts to adduct the thigh at the hip joint and flex and medially rotate the thigh [5]. This muscle also contributes to flexion of the thigh when the foot leaves the ground and begins to swing forward during running [14]. It is known that the psoas major muscle is a muscle for the hip flexion and that muscle volume/CSA of the psoas major is associated with sprint performance [1] [2] [4]. The adductor brevis may support the flexion and medially rotation of the thigh together with the psoas major, which may contribute to sprint running performance in female 100 m sprinters.

5. Conclusion

In conclusion, we found a correlation between adductor brevis muscle volume relative to body mass and 100 m season best time, although there was no correlation between total adductors muscle volume and sprint performance. These results suggest that the adductor brevis may play a role in superior sprint performance in female sprinters.

Acknowledgements

The authors would like to thank the individuals who voluntarily gave their time to participate in this study. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of Interest

The authors declare that they have no conflicts of interest relevant to the content of this study.

References

[1] Copaver, K., Hertogh, C. and Hue, O. (2012) The Effects of Psoas Major and Lumbar Lordosis on Hip Flexion and Sprint Performance. *Research Quarterly for Exercise and Sport*, **83**, 160-167. https://doi.org/10.1080/02701367.2012.10599846

[2] Hoshikawa, Y., Muramatsu, M., Iida, T., Uchiyama, A., Nakajima, Y., Kanehisa, H. and Fukunaga, T. (2006) Influence of the Psoas Major and Thigh Muscularity on 100-m Time in Junior Sprinters. *Medicine and Science in Sports and Exercise*, **38**, 2138-2143. https://doi.org/10.1249/01.mss.0000233804.48691.45

[3] Sugisaki, N., Kanehisa, H., Tauchi, K., Okazaki, S., Iso, S. and Okada, J. (2011) The Relationship between 30-m Sprint Running Time and Muscle Cross-Sectional Areas of the Psoas Major and Lower Limb Muscles in Male College Short and Middle Distance Runners. *International Journal of Sports and Health Science*, **9**, 1-7. https://doi.org/10.5432/ijshs.20100018

[4] Sugisaki, N., Kobayashi, K., Tauchi, K. and Kanehisa, H. (2018) Associations between Individual Lower Limb Muscle Volume and 100-m Sprint Time in Male Sprinters. *International Journal of Sports Physiology and Performance*, **13**, 214-219. https://doi.org/10.1123/ijspp.2016-0703

[5] Pansky, B. (1996) Review of Gross Anatomy. 6th Edition, McGraw-Hill, New York.

[6] Mann, R.A., Moran, G.T. and Dougherty, S.E. (1986) Comparative Electromyography of the Lower Extremity in Jogging, Running, and Sprinting. *American Journal of Sports Medicine*, **14**, 501-510. https://doi.org/10.1177/036354658601400614

[7] Montgomery, W.H., Pink, M. and Perry, J. (1994) Electromyographic Analysis of Hip and Knee Musculature during Running. *American Journal of Sports Medicine*, **22**, 272-278. https://doi.org/10.1177/036354659402200220

[8] Abe, T., Dankel, S.J., Buckner, S.L., Jessee, M.B., Mattocks, K.T., Mouser, J.G., Bell, Z.W. and Loenneke, J.P. (2019) Differences in 100-m Sprint Performance and Skeletal Muscle Mass between Elite Male and Female Sprinters. *Journal of Sports Medicine and Physical Fitness*, **59**, 304-309. https://doi.org/10.23736/S0022-4707.18.08267-1

[9] Abe, T., Kondo, M., Kawakami, Y. and Fukunaga, T. (1994) Prediction Equations

for Body Composition of Japanese Adults by B-Mode Ultrasound. *American Journal of Human Biology*, **6**, 161-170. https://doi.org/10.1002/ajhb.1310060204

[10] Brozek, J., Grande, F., Anderson, J.T. and Keys, A. (1963) Densitometric Analysis of Body Composition: Revision of Some Quantitative Assumption. *Annals of the New York Academy of Sciences*, **110**, 113-140. https://doi.org/10.1111/j.1749-6632.1963.tb17079.x

[11] Abe, T., Kearns, C.F. and Fukunaga, T. (2003) Sex Differences in Whole Body Skeletal Muscle Mass Measured by Magnetic Resonance Imaging and Its Distribution in Young Japanese Adults. *British Journal of Sports Medicine*, **37**, 436-440. https://doi.org/10.1136/bjsm.37.5.436

[12] Abe, T., Dankel, S.J., Buckner, S.L., Jessee, M.B., Mattocks, K.T., Mouser, J.G., Bell, Z.W. and Loenneke, J.P. (2019) Magnetic Resonance Imaging-Measured Skeletal Muscle Mass to Fat-Free Mass Ratio Increases with Increasing Levels of Fat-Free Mass. *Journal of Sports Medicine and Physical Fitness*, **59**, 619-623. https://doi.org/10.23736/S0022-4707.18.08683-8

[13] Ogawa, M., Yasuda, T. and Abe, T. (2012) Component Characteristics of Thigh Muscle Volume in Young and Older Healthy Men. *Clinical Physiology and Functional Imaging*, **32**, 89-93. https://doi.org/10.1111/j.1475-097X.2011.01057.x

[14] Wirhed, R. (2006) Athletic Ability and the Anatomy of Motion. 3rd Edition, Elsevier Health Sciences, Amsterdam, Netherlands.

International Journal of Clinical Medicine, 2019, 10, 477-522
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Pathogenic Mycoplasma Infections in Chronic Illnesses: General Considerations in Selecting Conventional and Integrative Treatments

Garth L. Nicolson

Department of Molecular Pathology, The Institute for Molecular Medicine, Huntington Beach, California, USA
Email: gnicolson@immed.org

How to cite this paper: Nicolson, G.L. (2019) Pathogenic Mycoplasma Infections in Chronic Illnesses: General Considerations in Selecting Conventional and Integrative Treatments. *International Journal of Clinical Medicine*, **10**, 477-522.
https://doi.org/10.4236/ijcm.2019.1010041

Received: September 12, 2019
Accepted: October 12, 2019
Published: October 15, 2019

Abstract

The presence of pathogenic mycoplasmas in various chronic illnesses and their successful suppression using conventional and integrative medicine approaches are reviewed. Evidence gathered over the last three decades has demonstrated the presence of pathogenic mycoplasma species in the blood, body fluids and tissues from patients with a variety of chronic clinical conditions: atypical pneumonia, asthma and other respiratory conditions; oral cavity infections; urogenital conditions; neurodegenerative and neurobehavioral diseases; autoimmune diseases; immunosuppressive diseases; inflammatory diseases; and illnesses and syndromes of unknown origin, such as fatiguing illnesses. Only recently have these small intracellular bacteria received attention as possible causative agents, cofactors or opportunistic infections or co-infections in these and other conditions. Their clinical management is often inadequate, primarily because of missed diagnosis, under- and inadequate treatment and the presence of persister or dormant microorganisms due to biofilm, resistence and other mechanisms. Pathogenic *Mycoplasma* species infections have been suppressed slowly by anti-microbial and integrative treatments, resulting in gradual reductions in morbidity, but not in every patient. Even if mycoplasmas are not a cause or an initial trigger for many chronic illnesses, they appear to play important roles in the inception, progression, morbidity and relapse of chronic illnesses in rather large patient subsets. Ignoring such infections can result in failure to achieve eventual patient recovery, even with application of potentially curative treatments.

Keywords

Chronic Diseases, Infections, Antibiotics, Herbel Therapy, Immune Enhancement, Membrane Lipid Replacement, Mycoplasma, Natural Supplements, Integrative Medicine

1. Introduction

Mycoplasmas belong to the class *Mollicutes*, and they are considered the smallest free-living prokaryocytes capable of self-replication [1] [2]. There are more than 200 bacterial microorganisms that belong to the genus of Mycoplasma, and the more than two dozen pathogenic species found in humans are typified by: 1) lack of an external cell wall; 2) obligate parasitic behavior; 3) intracellular growth; and 4) the loss of many of their genes due to reductive evolution [2] [3] [4]. Mycoplasmas are widely distributed in nature, where they are often found attached to the external surfaces of cells or residing and replicating inside host cells [1] [2].

It has been only fairly recently that mycoplasmas have been identified as important pathogens in humans, animals, plants and insects [1] [3] [5] [6]. There is evidence in humans that pathogenic mycoplasmas are associated with certain chronic diseases where they could function as causative agents, cofactors or opportunistic infections that cause patient morbidity [5] [6] [7]. For example, pathogenic mycoplasmas in humans are often associated with respiratory infections, urogenital infections, fatiguing illnesses, autoimmune diseases, neurodegenerative and neurobehavioral diseases and complications affecting the central nervous system, cardiac infections, oral infections, peridontal diseases, sexually transmitted diseases and systemic infections found in various solid cancers and leukemias and immunosuppressive diseases, such as HIV-AIDS [5] [6] [7].

Although various mycoplasmas are commonly found as commensals in the oral cavity and at other superficial sites [3], certain pathogenic species appear to cause morbidity when they penetrate into the blood and spread to and colonize various tissues [2] [5] [6] [7]. For example, *Mycoplasma hominis* and *Ureaplasma urealyticum* are common inhabitants of the human genital tract, but they can play an etiologic role in pyelonephritis, pelvic inflammatory disease as well as in post-abortion and post-partum fevers [3] [7] [8] [9] [10]. Furthermore, there are reports of mycoplasmas causing serious acute infections, such as septicemia, septic arthritis, neonatal meningitis and encephalitis [2] [6] [7] [11]. As an example of their pathogenic potential in mammals, it was shown that *M. fermentans* can cause severe neurological signs and symptoms after injection into the cerebral fluid of rats [12] [13]. Although still a subject of intense discussion, several pathogenic mycoplasma species have been proposed to be etiologic agents or cofactors in various chronic diseases of man [1] [2] [3] [5] [6] [7]. This will be discussed briefly in the next sections.

Mycoplasmas contain the smallest known self-replicating genomes, and they have an unusually low G + C content (25% - 33%) [1] [4]. With their limited genomes mycoplasmas have provided researchers with a simple model for the identification of the minimal gene set required for the survival and growth of a free-living bacterium [14] [15]. The small genomes of *M. genitalium* and *M. pneumoniae* encode approximately 400 - 600 proteins, compared to about 4000 in *E. coli* [16]. Furthermore, mycoplasmas still maintain all of the essential genes

for replication, transcription, and translation as well as the minimal number of energy metabolism genes needed for their parasitic modes of life. They can do this with a core number of slightly less than 400 essential genes [17].

Essentially all mycoplasmas live as parasites or commensals in various species of animals and plants, where they are usually found attached to or inside host cells [3] [4] [5]. Thus a significant number of mycoplasmal genes are devoted to encoding cell adhesion and attachment structures as well as variable membrane surface antigens to maintain parasitism and evade host immune and non-immune surveillance systems [3] [4] [5]. The adherence of mycoplasmas to specific tissue cell surfaces is a crucial step in the establishment of infections, and pathogenic mycoplasmas possess specialized structures that permit targeted cell attachment to specific host cells. For example, *M. pneumoniae*, which is commonly found in cases of atypical childhood pneumonia, requires a network of interactive adhesion molecules and accessory proteins for its adherence to host epithelial cells [4] [5]. The adhesion molecules must cluster at specific mycoplasma organelles in close association with cytoadherence-related accessory proteins that appear to function together and comprise a primitive membrane adhesion structure [4] [5] [18] [19].

Mycoplasmas can adapt quickly to their microenvironments. This adaptation is an important element in mycoplasma pathogenicity, and it can be attributed to their rapidly varying genomic structures and abilities to quickly change [3]. When mycoplasmas evolved and adapted to parasitic modes of life, their transformation was likely made possible by devoting many of their genes to parasitic functions. Thus the genetic evolutions of mycoplasmas have ensured rapid alterations in cell membrane characteristics, such as membrane lipid phase variations and variable regulations of distinct membrane surface proteins involved in cell colonization and host immune system avoidance. Some examples include size and sequence variations in the structural domains of surface proteins, epitope masking and demasking, and changes in protein surface presentations [20].

Mycoplasmas are known to variably express structurally heterogeneous cell surface antigens and adhesion molecules. For example, variations in the genes encoding cell surface adherence molecules, such as the variable adherence-associated (Vaa) antigen, reveal distinct patterns of mutations capable of generating multiple changes in mycoplasma cell surface antigen molecules and their antigenic size and diversity [21]. In addition, mycoplasmas can scavenge host structures, such as host glycans, for decoration of their own surface glycolipids to avoid detection [22].

Variable surface antigenic structures and rapid changes in their expression are thought to play important roles in the pathogenesis of mycoplasmal infections by providing altered epitope structures for an escape from immune responses and changes in adhesion structures. This can influence cell and tissue colonization and penetration of mucosal barriers [21] [23].

Mycoplasmas have small and unique genomes that contain repetitive and other elements, and this contributes to the variability in antigenic structures. For

example, the genome of *M. genitalium* was recently sequenced and found to encode a number of identifiable membrane proteins as well as membrane glycolipo proteins whose sequences do not resemble previously sequenced genes [24]. For example, repeated fragments of a gene encoding a 140 kDa adhesion lipoprotein (MgPa) have been found, and interestingly, this lipoprotein has been localized to the tips of mycoplasma protrusions where it facilitates cell attachment and penetration [25]. Repetitive sequence elements are also variably present that do not appear to encode expressed proteins. However, recombination of these repetitive elements with other genes may explain the appearance of polymorphisms within the genes and their encoded surface proteins of different mycoplasma strains. These repetitive elements, for example in the *M. genitalium* genome, may provide a reservoir of sequences that could contribute to the variability of antigenic structures and adhesive properties found in pathogenic mycoplasmas [26].

2. Mycoplasmas and Host Response Systems

Pathogenic mycoplasmas can activate or suppress host response systems, and they apparently use these and other strategies to evade host immune surveillance [27]. For example, pathogenic mycoplasmas can act as immune cell suppressors/activators and inhibit or stimulate the proliferation of various lymphocyte subsets involved in memory, suppression and other activities. Pathogenic mycoplasmas can also induce B-cell differentiation and trigger the secretion of pro-inflammatory cytokines, including interleukin-1β (IL-1β), IL-2, IL-6, IL-8, among others, tumor necrosis factor-α (TNFα), various interferons, and granulocyte macrophage-colony stimulating factor (GM-CSF) from cells. This also occurs *in vivo* in patients with pathogenic mycoplasmal infections. In fact, the release of inflammatory cytokines *in vivo* is predictive of refractory mycoplasmal infections in children [28].

Mycoplasma-derived lipopeptides can directly stimulate host response cells, such as macrophages. Such lipoproteins have been found to be highly effective at immune stimulation similar to endotoxins derived from other bacteria [29]. Using nitric oxide release by macrophages as an indicator of immune stimulation a *M. fermentans*-derived lipopeptide was identified as a potent activator of macrophage function [30]. In addition, *M. fermentans*-derived lipoproteins can interfere with the interferon gamma-dependent (IFN-γ-dependent) expression of MHC class II molecules on macrophages [31].

Pathogenic mycoplasmas are also able to secret soluble factors that can activate and stimulate proliferation or inhibit the growth and differentiation of immune competent cells. For example, *M. penetrans* can induce significant proliferative responses in peripheral blood mononuclear cells, and this was found to be associated with the expression of surface markers of lymphocyte activation. The activation was observed in lymphocytes (both CD4+ and CD8+ T lymphocytes) from healthy donors as well as from HIV-infected subjects at different stages of disease progression [32]. Thus pathogenic mycoplasmas have evolved with the ability to modulate and interfere with host responses.

The secretion of immune-modulating substances stimulated by pathogenic *Mycoplasma* species is an important aspect of mycoplasma immune modulation [27]. In the case of *Mycoplasma fermentans* a released lipoprotein can stimulate the induction of monocyte cytokines and chemokines [33]. Another example is spiralin, a well-characterized mycoplasma lipoprotein that can stimulate the *in vitro* proliferation of human peripheral blood mononuclear cells and murine splenocytes. This results in secretion of proinflammatory cytokines, such as TNFα, IL-1 and IL-6. Spiralin can also induce the maturation of murine B-cells. The spiralin-mediated activity appears to be similar to other immune-modulating lipoproteins secreted by other pathogenic bacteria [34]. The stimulation of various cytokines by pathogenic mycoplasmas is an important property that contributes significantly to patient morbidity.

Pathogenic mycoplasmas can evade immune recognition and destruction by undergoing rapid surface antigenic variations [7] [27]. Even with their slow intracellular growth rates, by rapidly altering their cell surface antigenic structures as well as modulating host immune responses, pathogenic mycoplasmas can evade host surveillance mechanisms [7] [27]. This helps explain the chronic nature of mycoplasmal infections and the inability of hosts to completely suppress pathogenic mycoplasmal infections via host responses that are effective against other more rapidly growing bacteria [27]. Slow-growing microorganisms like mycoplasmas use these properties to change surface antigens and hide from immune systems, a strategy that is quite unlike most rapidly growing bacteria that attempt to use their rapid proliferation rates to outpace and overwhelm host immune defenses.

Adaptations of pathogenic mycoplasmas to unique host microenvironments are usually accompanied by rapid changes in cell surface adhesion receptors (for cell binding and entry) as well as rapid structural protein changes in order to mimic host antigenic structures (antigen "mimicry"). During chronic pathogenic mycoplasma infections the size, antigenic diversity and expression of cell adhesion antigens change. For example, the divergence of variable surface antigens can affect the adherence properties of mycoplasmas and enhance their abilities to evade foreign protein recognition by host immune systems, thus contributing to their adaptive abilities and survival in their human hosts [27]. This is only one example of the abilities of mycoplasma surface antigens to promote survival and pathogenic properties [5] [7] [23] [27].

3. Mycoplasma Toxicity and Pathogenesis

As described briefly above, pathogenic mycoplasmas can induce host responses that result in the release of inflammatory cytokines that cause host symptoms. In addition, the severity of host symptoms during pathogenic mycoplasma infections parallels the elevated expression of inflammatory cytokines [21] [27].

Various virulence mechanisms have been implicated in the pathogenesis of mycoplasmas (reviewed in [21] [35]). For example, intracellular mycoplasmas compete for cellular nutrients and metabolites, and this can interfere with cellu-

lar metabolism by depleting biosynthetic precursors and disrupting metabolic and synthetic pathways. Mycoplasmas secrete some of their own enzymes, such as lipases, proteases, nucleases and other enzymes, that can disrupt and interfere with host substrates, structures, enzymes and metabolic cycles [5] [21]. Mycoplasmas also have the capacity to stimulate the generation of hydrogen peroxide and superoxide radicals that damage host cellular membranes and other structures [36]. There are other possible ways that pathogenic mycoplasmas could be involved in damaging host cellular structures and normal cellular processes, such as direct membrane-membrane interactions found in cell adhesion, membrane fusion, vacuolization, and release of toxins or cytopathic molecules, but the actual molecular damage mechanisms of many of these interactions have remained elusive [21] [35]. The goal of pathogenic mycoplasmas is to hide and survive, not necessarily to kill their host, and they are very adept at achieving these goals.

One property of pathogenic mycoplasmas that can be used to directly suppress host cell responses is the initiation of apoptosis or programmed cell death of particular host cells, such as cells involved in host immune and non-immune responses [35]. One of the hallmarks of this process is host cell DNA fragmentation. For example, *M. penetrans* can induce or enhance apoptosis of peripheral mononuclear cells. The usual telltale sign of this is DNA fragmentation (typified by DNA ladder formation seen after electrophoretic separation) catalyzed by endogenous Ca^{2+}, Mg^{2+}-dependent endonucleases. In this example, an *M. penetrans* endonuclease (p40) was identified as a pathogenic mycoplasma determinant [36].

Pathogenic mycoplasma-released nucleases may also be involved in secondary necrosis, as seen in some advanced mycoplasmal infections [37]. This is indicated by the occurrence of both morphological characteristics of apoptosis (chromatin condensation) and necrosis (loss of membrane integrity with organelle swelling) [38]. In these examples, cell death was accompanied by oligonucleosomal DNA fragmentation and loss of chromosomal DNA.

Cytokine-inducing activity is a general feature of most if not all pathogenic mycoplasma species, but it appears that only some mycoplasma species can induce cell death. In the case of *M. fermentans* infections the cell death-inducing effects were not mediated by known mycoplasmal-induced cytokines, which are typically mediated by lipid-associated molecules (lipoproteins). Also, they were not accompanied by decreases in the mitochondrial trans-membrane potential or inhibited by preincubation with the antioxidant drug N-acetylcysteine, events typically found in TNFα-mediated apoptosis. Instead, a non-lipid-associated protein (15 - 30 kDa) mediated the cytocidal effects [39].

Pathogenic mycoplasmas can cause cardiovascular and pulmonary manifestations that can result in extreme patient morbidity [35] [40]. There are several examples of this in the literature, and they have been reported as vascular occlusion due to thrombosis caused by stimulation of autoimmunity and the formation of vascular immune complexes. Vascular occlusion has been reported for

heart, lung, kidney, brain and other organs in pathogenic mycoplasma infections [38] [40].

In addition to stimulating cell and organ death, pathogenic mycoplasmas can also release growth inhibitory molecules into their surroundings. For example, the enzyme arginine deaminase is an example of a growth-inhibitory enzyme derived from mycoplasmas that inhibits the growth of human T-cells and T-lymphoblastoid cell lines. Arginine deaminase can suppresses IL-2 production and receptor expression in T-cells stimulated by non-specific mitogens, while also inducing the morphologic features of dying cells, including the type of DNA fragmentation seen during apoptosis [41]. This enzyme has been followed in patients with community-acquired pneumonia as a possible marker for *M. pneumoniae* infections [42].

Pathogenic mycoplasmas can also release toxins that directly damage cells or activate innate host response systems [40]. For example, Becker *et al.* [43] have isolated a *Mycoplasma pneumoniae*-released factor, called the community-acquired respiratory distress syndrome toxin (CARDS), an ADP-ribosylating and vacuolating cytotoxin. This pathogenic mycoplasma toxin activates the NLRP3 inflammasome complex and causes subsequent release of IL-1 and hyper-inflammation that can cause tissue damage and other pathologies. The mycoplasma toxin appears to cause pulmonary inflammation, cytokine release, and significant airway dysfunction and may be responsible, in part, for respiratory failure and fatal outcomes found in acute *M. pneumoniae* infections [44].

4. Mycoplasmal Infections in Various Acute and Chronic Illnesses

Pathogenic mycoplasmal infections have been found in a variety of human diseases and conditions [45] [46] [47]. First, pathogenic mycoplasmas have been detected at higher incidence in blood and tissue specimens obtained from patients with various chronic illnesses compared to comparable healthy controls. Since the possible involvement of mycoplasmas in the cause and pathogenesis of chronic illnesses have not been firmly established, it remains uncertain whether such mycoplasmas are causal agents, cofactors, or opportunistic infections or co-infections in patients with various diagnoses [7] [11] [45] [46] [47]. As stated above, various mycoplasmas can be found as normal flora in the genitourinary tract, oral cavity, gut and other sites, but they are not thought to be pathogenic at these superficial sites [7] [45] [46] [47].

The determining factor on whether mycoplasma infections are pathogenic in various diseases and illnesses or simply bystanders could be the requirement that pathogenic mycoplasmas must penetrate into the blood circulation and eventually enter into tissues and cells. This could explain the routine result of finding pathogenic mycoplasmas in the genitourinary tract, oral cavity, gut and occasionally in the blood of asymptomatic subjects. Unless mycoplasmas penetrate into the blood circulation and eventually enter tissues and cells, it may be un-

likely that they can exert their full pathogenic effects [6] [7] [45] [46] [47].

4.1. Laboratory Testing for Pathogenic Mycoplasmas

The clinical detection of *Mycoplasma* species has been achieved using *in vitro* culture, serology or molecular detection of DNA or RNA (reviewed in [40]). Although the first two approaches have been used extensively, they have many limitations. For example, the slow and fastidious growth of most *Mycoplasma* species precludes *in vitro* culture as a practical, reliable method of detection. Pathogenic mycoplasmas generally require intracellular conditions for growth, and this cannot be easily duplicated in culture. Serological testing is also difficult because of the possibility of suppression of immune responses and the sequestering of pathogenic mycoplasma antigens inside cells and away from the immune system. Thus more recent publications have used molecular techniques, such as various methods of polymerase chain reaction (PCR) for detection of *Mycoplasma* species [40].

However, there are still some problems with many of these methods due to specimen limitations, availability of clinical samples that contain pathogenic mycoplasmas, rapid sample degradation, the presence of inhibitors or interfering factors and other considerations. Some of the technical limitations have been extensively discussed by Waites *et al.* [40] and will not be further considered here.

Non-amplified DNA hybridization methods have also been used successfully in a few studies, for example for *M. fermentans* sequences [48]. In general, these test formats suffer from the complex nature of the tests themselves, their reliability and the requirement for sufficient numbers of microorganisms for a positive result.

In studies where both serology and PCR were used there were often differing results, depending on the time of sampling, antibody type (IgM, IgG, IgA, etc.) examined, PCR method used, and other factors [49]. New methods have been developed using, for example, matrix-assisted laser desorption ionization-time of flight mass spectrometry or other techniques, but these are time-consuming, costly and have not been thoroughly evaluated [40]. Thus there remain some questions on whether negative test results using currently and routinely available diagnostic procedures are truly reliable indicators that mycoplasmal infections are not involved.

4.2. Respiratory Infections

Pathogenic mycoplasmas have been routinely observed in community-acquired respiratory infections, such as atypical pneumonia [40] [50]. Indeed, *M. pneumoniae* represents one of the more common and potentially dangerous etiological agent found in interstitial pneumonia, and it is often associated with a long-lasting tracheobronchitis in children and adults [50] [51]. Indeed, this infection is often overlooked in adults, even though it is quite commonly found in

certain communities, such as in the military [52] [53]. In addition to the recognition of *M. pneumoniae* as a possible etiological agent in primary atypical pneumonia, it is also found in other inflammatory and autoimmune diseases, such as various forms of arthritis (to be discussed below).

Along with their ability to act as precipitators, cofactors or progression factors in severe respiratory diseases, pathogenic mycoplasmas have been found to facilitate alterations in local respiratory immunity, such as activation or suppression of pulmonary macrophages or T-cells. In addition, some mycoplasmas have the capacity to alter the structures and functions of host pulmonary cells, such as activation and inflammation of lung epithelium and endothelium, especially by secretion of cytokines, enzymes and other molecules [49] [50] [51] [52].

The pathogeneticity of *M. pneumoniae* is thought to be based, at least initially, on its adhesion to respiratory epithelial cells. Once attached to epithelial cells, its unique gliding motility and the induction of pathological hyper-stimulation of local host cellular response mechanisms appear to be important [19] [21] [35] [40]. During the acute phase of either primary or secondary infections by *M. pneumoniae* cytokines commonly associated with innate resistance and inflammation are expressed [28]. Host responses involving pulmonary macrophages, mast cells, neutrophils, natural killer cells, and T and B cells as well as humoral antibody responses have been extensively studied in pathogenic mycoplasma respiratory infections [27] [35] [40]. Thus the pathogenesis of *M. pneumoniae* lung infections is known to be associated with host responses and elevated expression of certain proinflammatory cytokines. It is also associated with extensive tissue damage and the expression of non-cytokine host factors [19] [21] [40]. In some patients this can result in a fatal course of the infection with multi-system involvement [54]. Tissue and cell damage caused by pathogenic mycoplasma-released enzymes and toxins were briefly discussed above, and several additional mechanisms have been described [39]-[44] [50].

Pathogenic mycoplasmas like *M. pneumoniae* have the capacity to mimic some of the structures of pulmonary host cells, and this could be important in allowing colonization and escape from host pulmonary immune recognition. For example, antigenic similarities between important functional adhesion molecules of *M. pneumoniae* and various host cell surface molecules could be one of the factors responsible for hindering host recognition and response mechanisms and could determine host failure to protect against repeated mycoplasma pulmonary colonizations [40] [50].

In addition to pneumonia, pathogenic mycoplasmas have for some time been implicated in the pathogenesis of chronic asthma [35] [40] [50]. Indeed, allergic sensitization and respiratory pathogens have been known for some time to be important in the inception of asthma [55]. Waites *et al.* have discussed the evidence for pathogenic mycoplasma infections in chronic asthma and its exacerbations [40].

4.3. Oral Infections

Various pathogenic mycoplasmas have been implicated in a variety of clinically important oral infections [6] [40]. Indeed, *Mycoplasma species* infections have been implicated in diseases of the gingival crevices and respiratory tract, and they have been found at high incidence in patients with gingivitis and periodontitis [56]. For example, mycoplasmas were cultured from almost all saliva and plaque samples in children with gingivitis [57]. Although their pathogenesis in oral diseases has not been well studied, various pathogenic mycoplasma species are able to induce cytokine secretion by gingival fibroblasts. Although mycoplasmas have been isolated using throat swabs, sputum and tracheal secretions, they are also routinely found in the oral cavities of normal hosts where they apparently do not cause symptoms. Thus the rather common finding of mycoplasmas, even pathogenic mycoplasmas, in the oral cavities of non-symptomatic subjects may simply reflect their superficial colonization of these sites. Unless accompanied by other pathogenic microorganisms, or if tissue damage occurs that allows entry of the mycoplasmas into surrounding tissues and blood circulation, they may not routinely express their full pathogenic potentials [6] [7].

4.4. Airway Inflammation

Pathogenic mycoplasma respiratory tract infections can result in airway inflammation and induction of bronchial hyper-responsiveness (BHR) [58]. The production of *M. pneumoniae*-specific IgE and IgA may play an important role in exacerbation of BHR and asthma. Elevated IgE antibodies specific to *M. pneumoniae* have been detected in the serum of children with *M. pneumoniae* pneumonia, and in patients with preexisting asthma-BHR increased levels of *M. pneumoniae* specific IgE and IgA occurred after infection (reviewed in [59]). Mycoplasmal infections are also routinely involved in severe asthma-BHR attacks in adults. For example, *M. pneumoniae* was isolatable in 24.7% of patients with asthma but in only 5.7% of control subjects, suggesting that this pathogenic mycoplasma plays a role in asthma attacks [60].

Although pathogenic mycoplasma infections are often associated with chronic asthma, the exact role of mycoplasmas in the pathogenesis of asthma remains unclear [59]. Mycoplasmas are likely only one of many agents that can trigger BHR, and other co-infectious or chemical agents may contribute to a complex disease process.

4.5. Urogenital Infections

Pathogenic mycoplasmas are commonly found in urogenital infections, such as *M. genitalium, M. fermentans, M. hominis, M. pneumoniae* and *Ureaplasma urealyticum* [61]. Importantly, *M. hominis* and *U. urealyticum* have been implicated in a wide variety of urogenital diseases, such as pelvic inflammatory disease, infertility, non-gonococcal urethritis (NGU) and other genital infections. Pathogenic mycoplasmas have been isolated from various tissues of patients,

such as urethra, fetal-placental tissue, cervix, endometrium, vagina, various wounds, and from urine, blood, peritoneal fluid, and amniotic fluid. *M. hominis* was one of the first mycoplasmas to be isolated from urogenital infections, and this species has been strongly associated with bacterial vaginosis [62]. The sequence of appearance of various pathogenic mycoplasma species in bacterial vaginosis may be a result of pathophysiological alterations of the vaginal ecosystem, and mycoplasmas appear to play an important role in this process. Pathogenic mycoplasmas are but one class of many types of bacterial infections that can be present at various times and in varying amounts in the vaginal ecosystem.

There is good evidence, however, for an etiological role for *U. urealyticum* in acute NGU and its chronic forms in men. This species was found in a majority of male patients with NGU, and sexually transmitted *U. urealyticum*, *M. hominis* or *Chlamydia trachomatis* infections have been detected in approximately one-half of 400 young symptomatic females under the age of 30, and a majority of 86 pregnant women with sexually transmitted diseases. Although the incidence rate of the detected microorganisms varied in different reports, infections with pathogenic mycoplasmas are thought to be an important cause or cofactor in many patients with urogenital conditions, such as inflammations, fertility problems and premature deliveries.

Some commonly found pathogenic mycoplasmas are not usually associated with NGUs. For example, *M. fermentans*, *M. penetrans*, and *M. pirum* were not found in urethral swab specimens collected from male patients with or without acute NGU. Although *M. fermentans* was not detected by PCR or culture methods in patients with urethritis or cervicitis, this species was detected by PCR in 4 of 232 amniotic fluid samples tested. The results suggest that in these four cases *M. fermentans* was transferred transplacentally. Histological evidence of chorioamnionitis was present in two of the four patients, a finding that supports the hypothesis that *M. fermentans* may be a possible cause of chorioamnionitis.

4.6. Infertility and Spontaneous Abortion

Pathogenic mycoplasmas are known to reduce fertility rates [63]. For example, *U. urealyticum* was found to be involved in a number of patients with fertility problems. This has been related to both women and men with these infections, and successful treatment has been correlated with improvements in fertility. For example, in men *U. urealyticum* infection was found to reduce sperm motility and viability, which are directly related to fertility, and treatment of this infection resulted in improvements in quantity, motility, and appearance of sperm, and importantly, in improvements in fertility [64]. Multiple studies on several species have shown that pathogenic mycoplasma infections are a risk factor for male infertility [65].

The mere presence of pathogenic mycoplasmas on, for example, the vaginal mucosal surfaces of the cervix or vagina is not necessarily an indication of infertility, because *U. urealyticum* and *M. hominis* may occur in 40% to 80% of asymptomatic women. However, where inflammatory infections of the upper

urogenital tract occur and are related to mycoplasmal infections, there was a good correspondence with infertility [64]. Although infections of the lower genital tract are not well correlated with infertility and adverse pregnancy outcomes, pathogenic mycoplasma infections of the upper tract and chorioamnion were strongly associated with adverse pregnancy outcomes [66]. The evidence in experimental animal infections is even clearer. In these cases Koch's postulates have been fulfilled. In addition, a portion of infertile clinical cases of nongonococcal urethritis have been confirmed as caused by pathogenic mycoplasmas [64].

Pathogenic mycoplasmal infections also cause spontaneous abortions and a higher risk of birth complications, such as post-Cesarean delivery endometritis [64]. Pathogenic mycoplasma infections appear to be important in prematurity, pregnancy loss, chorioamnionitis and other complications. These microorganisms can invade the amniotic cavity and cause intense inflammatory reactions in the absence of labor [66].

4.7. Immunosuppressive Diseases

One of the most immunosuppressive diseases in humans is caused by the human HIV-1 virus. Various *Mycoplasma spp.* have been implicated as infectious cofactors along with HIV-1 virus in the pathogenesis of HIV-AIDS [67] [68]. Using relatively insensitive techniques three mycoplasma species have been detected in patients with HIV-1 infections, and the incidence of systemic mycoplasmal infections in HIV-AIDS patients is likely much higher than previously thought. Possible mechanisms that could allow mycoplasmas to influence HIV pathogenesis appear to be the specific and direct activation or suppression of the immune system, the production of superantigens with subsequent alterations in immune responses, and their contribution to the oxidative stress observed in HIV-positive patients [68] [69].

Specific species of mycoplasmas have been isolated from HIV-infected individuals. *Mycoplasma fermentans* was the first mycoplasma species reported in AIDS patients, and there is evidence that it is an important cofactor in the progression of AIDS [70]. *M. fermentans* co-infection occurs with HIV-1 infections in approximately one-half of patients with AIDS [71], and lower percentages of other pathogenic mycoplasma species have also been identified [72] [73].

Mycoplasma molecular mimicry may be involved in the pathogenesis of AIDS. Antigen similarities between the surface components of pathogenic mycoplasmas and HIV-1 have led to speculation that they use similar mechanisms for cell entry. Interestingly, the HIV-1 gp120 envelope glycoprotein and *M. genitalium* adhesin proteins share sequence homology and also have significant similarity with the CD4-binding site of the class II major histocompatibility complex (MHC) proteins. The interactions of pathogenic mycoplasmas with MHC-related antigens on host cells could contribute to a number of possible outcomes, including T-cell dysfunction, T-cell depletion, T-cell shift, B-cell proliferation, hyperglobulinemia and antigen-presenting cell dysfunction. All of

these have been observed during the development and progression of HIV-AIDS [74].

4.8. Rheumatic Diseases

Although the underlying causes of rheumatic diseases, for the most part, remain unknown, these diseases appear to involve, at least in part, infectious agents [75] [76]. Also, the progression of rheumatic diseases may be related to infectious processes. The clinical and pathological similarities between known infectious diseases in animal species and those of some human rheumatic diseases, such as rheumatoid arthritis, have supported microbial etiologies. In fact, there is a long list of microorganisms, including aerobic and anaerobic bacteria, as well as viruses, that have been proposed as important in these illnesses. Among these possible infectious agents are various species of pathogenic mycoplasmas [6] [75].

There is increasing evidence that pathogenic mycoplasmas may promote the progression of rheumatic diseases, such as rheumatoid arthritis (RA) [6] [75]. First, mycoplasmas have been proven to cause arthritis in animals [77]. Second, various pathogenic species of mycoplasmas have been detected in the synovial fluid and blood of RA and other arthritis patients [78] [79]. Although a few reports have not confirmed this, possibly because of the insensitive techniques used for detection, various species of pathogenic mycoplasmas have been localized in the joint tissues of patients with arthritis [59] [78] [80].

Pathogenic mycoplasmas are known to be able to induce immune dysfunction and autoimmune reactions that could be related to the development of arthritis [21] [40] [45] [59]. Thus, mycoplasmal infections could be, in part, involved in the pathogenesis of RA [20] [40] [78]. In animal models of arthritis, *M. arthritidis*-related superantigens were found to compromise T-cells, and they can trigger and exacerbate autoimmune arthritis. These superantigens involve parts of a T-cell receptor that has been proposed to be involved in arthritis [81]. Furthermore, mycoplasmas can release substances, such as oxygen free radicals and chemotactic and aggregating substances that could interact with mononuclear and other cell types.

Importantly, the injection of isolated membranes from *M. arthritidis* resulted in toxicities in animals similar to those found in rheumatic disease [82]. This might be related to correspondence in the antigenic epitopes of chondrocytes and *M. arthritidis* membrane components. Similarly, using immunological methods the presence of trace amounts of pathogenic mycoplasmal antigens or specific antibodies against pathogenic mycoplasma species were found quite often in RA patients [82].

4.9. Cardiovascular Diseases

Infections of the cardiovascular system by pathogenic mycoplasmas have been reported recently in patients with different forms of carditis [40] [59]. This has

also been found in chronic *M. pneumoniae* infections [59]. Indeed, endocarditis and myocarditis associated with *M. pneumoniae* infections appear to be important causes in the fatal outcomes in *M. pneumoniae*-infected patients [83]. Direct invasion of *M. pneumoniae* into pericardial tissue appears to be the likely cause of pericarditis, rather than autoimmune phenomena [83].

4.10. Autoimmune Diseases

Pathogenic mycoplasmas seem to play an important but not well-understood role in many autoimmune diseases. Several characteristics of mycoplasmas make them attractive as agents that could be responsible for triggering autoimmune responses. First, during their intracellular replication and release from host cells mycoplasmas can capture antigens from the host cell surface and incorporate them into their cell membranes. This can lead to concomitant immune responses against these host antigens and possibly autoimmune reactions. Second, mycoplasmal antigens often mimic host antigens. If immune responses are generated against these mycoplasmal antigens, the result could be cross-reactivity to normal host antigens. Third, mycoplasmas can cause apoptosis of host cells with subsequent release of normal host antigens that could elicit host autoimmune responses.

There is an example of a pathogenic mycoplasma superantigen immunomodulator that can be found during *M. pneumoniae* infections and cause transient autoimmune hemolytic disorders characterized by high titers of autoantibodies, in this case against carbohydrate antigens [84]. The effects of super-antigens on immune systems may be elicited through their binding to the outer portions of MHC structures on antigen-presenting cells and to the non-antigen recognizing structures of the hypervariable regions of T-cell antigen receptors [81]. Such super-antigens can also induce immunological tolerance [81]. When injected into mice, *M. arthritidis* super-antigen causes a chronic arthritis that resembles RA histologically. This mycoplasma species produces a super-antigen (MAM) that in humans preferentially expands T-cells whose antigen receptors (TCR) express the segment Vbeta17. T cells with this phenotype appear to be increased in frequency in RA synovial effusions. In addition to resembling human RA pathologically, other diseases, such as Kawasaki disease, Sjögren syndrome, lupus erythematosus and multiple sclerosis also show oligoclonal expression of TCR beta specificities on infiltrating T-cells [85].

4.11. Neurodegenerative and Neurobehavioral Diseases

Infections have been recently considered to be important in neurodegenerative and neurobehavioral diseases [45] [46] [47] [86]. Infectious bacterial agents, such as *Mycoplasma spp.*, *Chlamydia pn.*, *Borrelia spp.*, among others, can enter the central nervous system (CNS) by direct penetration of the blood-brain-barrier, intraneuronal transfer, or by being carried inside infiltrating mononuclear cells [46] [86]. Once inside the CNS such infections appear to be common features of various neurodegenerative conditions that cause dementia and other brain im-

pairments [46]. Neurobehavioral disorders overlap with neurodegenerative diseases in their signs and symptoms and can be found in brain disorders of mainly the elderly, such as stroke, dementia, multiple sclerosis and other disorders, as well as disorders of the young, such as autism spectrum disorders, attention deficit disorders and other brain developmental impairments. Both types of conditions are poorly understood and generally poorly addressed clinically [87].

To understand the complexities of neurodegenerative and neurobehavioral disorders there are a variety of considerations, including genetics, immune functions, head trauma, nutritional deficiencies, mitochondrial defects, oxidative stress, environmental toxins, heavy metal and chemical exposures, neurotransmitter abnormalities and chronic viral and bacterial infections [45] [46] [47] [86] [87]. Here we will only consider the potential role of chronic pathogenic mycoplasmal infections in these diseases.

A major adult onset neurodegenerative disease, amyotrophic lateral sclerosis (ALS), is an example of a progressive CNS disease that ultimately results in death, usually by paralysis and respiratory failure due to destruction of upper motor neurons in the cortex and lower motor neurons of the brain stem and spinal cord [88]. Evidence for the presence of particular viruses and intracellular bacteria in the brains of ALS patients has stimulated explorations for various pathogens [45] [46]. In fact, pathogenic mycoplasmal infections were found to be quite common in veterans and civilians with ALS, with 83% of ALS patients showing positive blood results compared to less than 9% of controls [89]. Some of these ALS patients also were positive for *Borrelia* infections. All of the Gulf War veterans with ALS that were tested were mycoplasma-positive, and almost all had *M. fermentans* infections [89]. Many patients with an incomplete diagnosis of ALS were also positive for mycoplasmal infections [46]. A recent study found that 46% of civilian ALS cases were positive by PCR [90].

Another rather common neurodegenerative disease, multiple sclerosis (MS), also shows evidence of chronic viral and bacterial infections, but in this case mycoplasmal infections were rarely found in brain tissue [91]. A somewhat different result using a different test source was obtained with female MS patients in remission. These patients were found to have higher levels of *M. pneumoniae* antibodies in their serum, suggesting that mycoplasmal infection(s) may be, in fact, related to progression in certain MS patients [92]. A more common finding was the presence of *Chlamydia pn.* in MS patients [93]. In MS the presence of various viruses, including human retroviruses, has shifted the focus to now look for endogenous viruses [94].

Neurobehavioral disorders, such as autism spectrum disorders (ASD) in children, also show high frequencies of chronic bacterial and viral infections. Several *Mycoplasma* species, *Chlamydia pneumoniae*, and human herpes-6 co-infections were found in the blood of a majority of children with ASD [95]. An association has also been found between ASD and *Borrelia burgdorferi* infections [96]. This has resulted in a proposal for long-term antibiotic therapy for children with ASD and associated conditions [97].

4.12. Fatiguing Illnesses

The most common fatiguing illness is chronic fatigue syndrome (CFS) or myalgic encephalomyelitis. This is an unexplained, long-term, persistent illness characterized by disabling fatigue plus additional signs and symptoms [98] [99]. Most if not all patients with CFS show evidence of chronic viral and bacterial infections (reviewed in [45] [47]). In fact, the odds ratio for the presence of chronic infections was calculated to be 18.0 ($p < 0.001$), suggesting that CFS patients have a very high probability of multiple chronic infections [100]. The most commonly found infections (by PCR of blood monocytes) were various pathogenic species of mycoplasmas [100] [101]. *M. pneumoniae* was the most common mycoplasma species found, followed by *M. fermentans, M. hominis*, and *M. penetrans* [101]. In European CFS patients these infections were also found, but in different frequencies. *M. hominis* was the most commonly found pathogenic mycoplasma in European CFS patients, suggesting that there are regional differences in the pathogenic mycoplasmal infections found in CFS patients [102]. In CFS patients the number of different chronic infections found in patients may also be important, because patients with multiple infections have a more severe illness course, in terms of the severity of their signs and symptoms [103].

A related fatiguing illness, fibromyalgia (FM) has many of the signs and symptoms of CFS, but its characteristic symptoms are diffuse widespread pain and other symptoms [104]. FM is often diagnosed along with CFS, and these two illnesses generally have overlapping signs and symptoms. However, widespread pain is the most characteristic symptom of FM, and it has been described as spontaneous pain, burning pain, pressure pain, and combinations of these [104]. Infections are a suspected trigger in FM, and various viral and bacterial infections have been found in FM patients (reviewed in [104]). Among the most frequent infections found are various pathogenic species of *Mycoplasma* [47] [101].

Another fatiguing illness is Gulf War Illness (GWI) [48] [89] [105]. GWI loosely fits the symptom profile of CFS, but depending on the multiple environmental exposures of veterans during this conflict symptoms can vary and include fatigue, pain, cognitive problems, gastrointestinal symptoms and respiratory and skin problems [105] [106]. This group of illnesses is thought to be caused by multiple hits, mainly through chemical and biological exposures. Many if not most GWI cases show evidence of pathogenic mycoplasmal infections (in about one-half of cases), and the majority of these were found to be *M. fermentans* infections [48] [89]. In fact, treating such infections with long-term anti-microbial therapy resulted in recoveries, but not with every patient [48] [105].

4.13. Other Illnesses and Conditions

There are a variety of other illnesses and diseases where pathogenic mycoplasmal infections can cause morbidity [45] [46] [47]. For example, Guillain-Barré syn-

drome, characterized by muscle weakness, pain, numbness, tingling in the arms, face and legs, among other symptoms, is a demyelinating neuropathy often associated with bacterial infections [107]. *M. pneumoniae* infections are commonly found in over one-half of Guillain-Barré syndrome patients [108]. As discussed above, *M. pneumoniae* infections are common in children, and they can be present as an important co-infection with other bacteria. As an example of this, pathogenic mycoplasma co-infections are often found in infectious illnesses like PANDAS (pediatric autoimmune neuropsychiatric disorders associated with Strepococci) [109]. Another PANDAS-related condition, Tourette's syndrome, has also been related to the involvement of bacterial co-infections, such as *Borrelia spp*. and *Mycoplasma pn.* [110] [111].

Infections like pathogenic mycoplasmas have also been found associated with a number of cancers, such as lymphomas [112], oral carcinomas [113], prostate cancers [114] and lung cancers [115]. Mycoplasmal infections can promote malignant transformation *in vitro* [116] and *in vivo* [117].

Various systemic illnesses involving different tissues and organs, such as vasculitis, dermatitis, neuritis, hepatitis, sarcoidosis, encephalitis, pancreatitis, hematologic illnesses, among others, can apparently be caused or promoted by pathogenic mycoplasma infections. Matsuda has listed a number of other conditions and illnesses that are linked to mycoplasmal infections (see Figure 1 [118]).

5. Conventional Treatment of Pathogenic Mycoplasmal Infections

Pathogenic mycoplasmal infections can occur alone or as co-infections with other microorganisms. Most often they are present with other bacteria, viruses, fungi, etc. in complex multiple infections, as discussed in various sections above. In many cases mycoplasmal infections are not the definitive infection that defines the condition. An example of this is chronic Lyme disease, a complex clinical condition with *Borrelia* species as the prominent infectious agent but with other bacterial, parasite, and viral components as co-infections [47] [119] [120] [121]. Pathogenic mycoplasmal co-infections are important in such multiple infection diseases, being present in up to 80% of chronic Lyme diseases cases [120] [122]. These multi-infectious conditions have been called Multiple Systemic Infectious Disease Syndromes (MSIDS) [121], and they present a significant treatment challenge to practitioners due to the number of infections involved, the wide variety of symptoms found, and the difficulty of finding effective treatments for complex clinical conditions [119] [120] [121].

5.1. Antimicrobial Treatments

There remains an ongoing controversy whether to treat clinically patients who have mycoplasmal infections. Even with mycoplasmal involvement as possible causes of chronic illnesses, as co-factors or as bystanders causing co-morbid conditions, this controversy has endured [45] [47] [123]. These notions will not

be considered here, but the most important concept for practitioners and patients is whether recovery from complex chronic illnesses with infectious components can be affected without taking into account the extensive morbidity that can be caused by pathogenic mycoplasmas. Treatment or not of such infections present in many chronic illnesses may be among the most important decisions that practitioners render for their chronically ill patients.

The conventional antimicrobial treatments of pathogenic mycoplasmal infections usually involve systemic therapy with oral antibiotics, but the choice of antibiotic(s) depends to a certain degree on the mycoplasma species being treated. Since mycoplasmas do not have a cell wall, antibiotics that act on cell wall synthesis are ineffective [2] [3] [7] [40] [50] [59] [124] [125]. Instead, mycoplasmas are treated with anti-microbials that attack their metabolism, replication, synthetic machinery or other specific bacterial targets. Since most mycoplasmas and ureaplasmas are generally sensitive to tetracyclines (doxycycline, minocycline, among others), with some notable exceptions, these should be considered for frontline treatment, and quinolones (ciprofloxacin, sparfloxacin, levofloxacin, ofloxacin, among others) [125] [126] [127] [128], as alternative treatment. However, *M. pneumoniae* and *M. genitalium* strains are especially sensitive to macrolides (azithromycin, clarithromycin, erythromycin, among others), whereas *M. hominis* strains are usually resistant [126] [127] [128]. Ureaplasmas are moderately susceptible to macrolides [127] [128]. *M. hominis* and *Ureaplasma urealyticum* are generally more resistant to tetracyclines than other species [129] [130], and *M. hominis* strains have been observed to be resistant to quinolones [131]. Some discussion of these antimicrobials and their uses in treating pathogenic mycoplasmal infections in chronic illnesses can be found in [132] [133] [134].

Mycoplasmal infections have been treated with intravenous antibiotic therapy, but this is generally done for only a short period of time due to potential long-term toxicities [132]. Treatment of pathogenic mycoplasma infections with oral antibiotics generally involves daily or pulsed treatment, such as every-other-day administration, at the maximum dose recommended for a particular antibiotic [132] [133] [134] [135]. Due to the cyclic nature of mycoplasmal proliferation some organizations recommend every-other-day antibiotic regimens [135].

There are important considerations in determing treatment, such as patient age. For example, some suggest that macrolides be considered first for young children with pathogenic mycoplasma infections due to the potential side effects of tetracyclines and quinolones [136]. However, some adverse effects, such as staining of developing teeth in children under the age of eight with tetracyclines, are not much of a problem with some newer antibiotics, such as doxycycline, and in some cases low-dose administration has been used [137].

Another important consideration is antibiotic resistance, which can occur during treatment [132] [138]. A major problem has been the shifting minimum inhibitory dose concentrations required to treat mycoplasmal infections with

antibiotics, such as treatment of *M. genitalium* infections with oral tetracyclines [139]. This requires increasing dose levels or shifting to a different antibiotic regimen [132].

In most chronic illness patients pathogenic mycoplasma infections do not respond quickly to anti-microbial therapy, so long-term therapy must be considered [123] [132] [133] [135]. Although acute infections involving *Mycoplasma* species, such as atypical pneumonia [50] [124], have been treated with short periods of antibiotics, this approach is generally ineffective for chronic infections [45] [46] [47] [48] [121] [123] [132] [133]. A major concern during long-term antibiotic treatment is the possibility of antibiotic resistance [140]. This can occur for a number of reasons that are connected to various genes or resistance determinants that, in turn, are linked to various targets and processes, including those that control the metabolism, activation-inactivation, influx and efflux of drugs and other important activities [140]. In general, this can be avoided, when necessary, by switching to another antibiotic [132].

5.2. Jarisch-Herxheimer Reactions

When antibiotics are used to treat pathogenic mycoplasmal infections, Jarisch-Herxheimer reactions (J-H reactions) usually occur [132] [141]. These are observed as temporary increases in the severity of signs and symptoms, and J-H reactions generally involve fevers, chills, muscle aches, fatigue, skin rashes, pain and other signs and symptoms related to cytokine release [141]. Although there are some rather simple methods to reduce the severity of some J-H reactions [132], their appearance with antimicrobial treatments is thought to be due to the release of mycoplasma particles and fragments and subsequent host response and cytokine release. Strong J-H reactions may suggest the efficacy of treatment, but the lack of strong J-H reactions during anti-microbial treatment is not necessarily an indication of treatment failure.

The signs and symptoms of J-H reactions during antibiotic therapy usually revert within days from their initial appearance but can go on for some time [132]. A simple approach to reducing J-H reactions may require reducing temporarily the dose of anti-microbial therapy, or rotating therapy, such as changing to antibiotics that display a completely different mechanism of action. In addition, drugs that decrease cytokine production or release can be effective, such as low-dose naltrexone and Cox-2 inhibitors [121]. The use of integrative treatments to mitigate J-H reactions will be discussed in section 6.4.

5.3. When to Stop Anti-Microbial Therapy

Other important considerations during the treatment of pathogenic mycoplasma infections are the overall length of treatment or when to stop therapy and how to deal with interfering microenvironments. Conventional guidelines suggest using only limited treatments for microbial infections, but such limited treatments in many chronic illnesses usually do not resolve the illness or the infection [133]

[142] [143]. Indeed, pathogenic mycoplasmas are slow-growing, cyclic, fastidious, intracellular microorganisms that appear to be less sensitive to antibiotics in their usual *in vivo* microenvironments [120] [121] [132].

In many but often less than a majority of patients with pathogenic mycoplasmas the infection can be suppressed by short-term antibiotic treatments of a few weeks, but this usually does not completely resolve the infection, and slow relapse often occurs. Thus the *in vitro* antimicrobial sensitivities of various mycoplasmas may not be useful in determining the time of treatment regimens *in vivo*, especially because of differing mycoplasma tissue locations and differences in metaboic states. Importantly, routine anti-microbial treatments may not accurately predict the time required to completely suppress systemic mycoplasmal infections in their natural intracellular microenvironments [143]. In most patients this has required prolonged treatments that have resulted in very slow recoveries, often requiring a year or more of treatment [48] [121] [123] [132] [133].

There are a number of factors *in vivo* that protect pathogenic mycoplasmas from antimicrobial therapies. For example, microbial biofilms may protect some mycoplasmas from antibiotics, allowing these mycoplasmas to survive in the presence of high drug concentrations, a characteristic that has been termed "recalcitrance" [143]. This will be discussed further in section 6.3. The intracellular locations and microenvironments of pathogenic mycoplasmas present additional membrane barriers for conventional drugs. Also, cells naturally try to detoxify chemicals that enter intracellular compartments. In addition, the presence of "persister tolerant" microorganisms, such as metabolically inactive forms, insures that there will always be some survivors after successful antimicrobial therapy. This is thought to occur, in part, by the dormancy of some microorganisms and diminishing antibiotic targets because of reduced metabolism and proliferation variants, along with selective pressures that can account for unusual antibiotic tolerance in certain survivor microbes [144].

There is also the possibility that host immune responses are essential in surveying survivor mycoplasmas that resist antimicrobial treatments. Variant microorganisms can resist surveillance by alteration or suppression of host responses [5] [6] [7] [19] [21] [144]. These and other possibilities could explain why lengthy treatments of antibiotics are required in most chronically ill patients to achieve complete mycoplasma suppression (without complete eradication) and allow patient recovery [7] [48] [132] [133]. Antibiotic therapy alone has not been successful in suppressing mycoplasmal infections in many patients without other treatment considerations [132] [133]. These will be discussed in Section 6.

There are some alternative procedures that can increase the *in vivo* effectiveness of antimicrobial therapies. One method that has been used to increase the effectiveness of antibiotics has been the use of agents that increase the penetrability or the intracellular activities or effectiveness of antibiotics or other drugs.

For example, the anti-malarial drug Plaquenil (hydroxychloroquine) has been used to alkalize intracellular compartments and improve antimicrobial entry and cytotoxic effects [121] [132] [145].

5.4. Anti-Inflammatory and Other Treatments

Inflammation can be a major issue in pathogenic mycoplasma infections, especially when multiple infections are involved [121]. Inflammatory cytokines are produced and released during mycoplasmal infections [28] [33] [35], and the levels of inflammatory cytokines have been correlated to patient morbidity [138]. In patients with severe mycoplasma infections inflammation can be serious, and anti-inflammatory treatments have been recommended [146]. For example, in children with severe *M. pneumoniae* infections corticosteroid treatment was temporally associated with clinical and radiographic improvements, and this anti-inflammatory addition to therapy was considered important in reducing patient morbidity [146].

In pathogenic mycoplasma cases with acute, severe inflammation patients have been treated with steroids or other immunosuppressive drugs with or without intravenous administration of immunoglobulins (IVIG) [134]. In the most refractory cases patients have received plasma exchange along with immunosuppressive drugs, but IVIG and plasma exchange remains a good option [147] [148].

Some antibiotics have immunomodulatory effects themselves. For example, tetracyclines and macrolides show some immunomodulatory effects in patients [149] [150]. This may have some advantage by reducing the inflammation associated with pathogenic mycoplasma infections.

There are a number of other considerations for patients with pathogenic mycoplasmal infections, such as allergies, food sensitivities, functional deficiencies, mitochondrial dysfunction, heavy metal and chemical sensitivities, mold and other biotoxins, endocrine and sleep disorders, nervous system dysfunction, pain and other phenomenon that make recovery difficult [121] [132]. Some of these considerations will be discussed in the next section. They are discussed in more detail elsewhere by Horowitz [151]. For the most part integrative treatment considerations are compatible with conventional therapies, and they are often used in conjunction with conventional therapies. Patients can generally continue their dietary supplements and foods while on antibiotics, but most antibiotics should not be taken at the same time of day as dietary supplements [132].

6. Diet and Integrative Treatments of Mycoplasmal Infections

As briefly discussed above, treatment of mycoplasmal infections with anti-microbials, such as long-term antibiotics, without other treatment considerations has, in general, been only marginally effective in achieving stable patient

recoveries [121] [133] [151]. Because of this, moreover, some patients have been inappropriately diagnosed with psychological problems due to their partial or incomplete recoveries [47]. Although the literature is rife with the appearance of pathogenic mycoplasmal infections in psychiatric disorders [47] [134], the persistence of psychiatric symptoms should not be considered the basis of an inability to recover using conventional anti-microbial treatments. Thus the addition of proper diet and integrative medical management along with conventional anti-microbial treatments should be considered, instead of assuming that patients have psychological reasons for not recovering on conventional antimicrobial therapies [131] [132] [133] [151].

6.1. General Nutritional Considerations

There are some basic nutritional or dietary considerations for patients undergoing treatment for pathogenic mycoplasma infections [132] [133] [151] [152] [153]. First, avoidance of high-sugar, yeast-containing and processed foods is essential [132] [153]. Decreasing or eliminating simple or refined sugars, alcohol, acid-forming, high-yeast- and trans-fat-containing foods as well as increasing intake of cruciferous vegetables, fish, soluble fiber foods and whole grains are useful for maintaining healthy digestive and immune systems [151] [152] [153].

Pathogenic mycoplasmas deplete many normal cellular molecules, mainly because they have limited genomes and can't synthesize many of the metabolites that they require for growth and proliferation [1] [5] [7] [15] [17] [153]. Restoring depleted nutrients is an essential part of any mycoplasma treatment program [132] [151] [152] [153]. Thus organizing diets to replace mycoplasma-depleted nutrients is especially important [153]. In the next sections consideration of important vitamins, minerals, lipids, proteins and other nutrients will be discussed.

Since fungal (yeast) overgrowth can be a problem, especially during antibiotic therapy, anti-fungal foods and supplements (grapefruit extract, olive leaf extract, garlic extract, berberine, and oregano oil) can be useful in treating or avoiding fungal infections [151] [152] [153]. Simple dietary advice for fungal or yeast overgrowth includes eliminating or significantly reducing yeast-containing and fermented foods, sugars, and alcoholic beverages [151] [152]. Anti-fungal drugs, such as nystatin and diflucan, can also be useful in controlling fungal/yeast infections [132] [151], but careful monitoring and consideration of possible inflammation should be done to improve outcome [151].

Controlling inflammation during therapy through dietary practice is a simple, practical approach. Recommendations include switching to "alkaline" or "Mediterranean" diets that are low in sugar, dairy, and processed meats and that are high in low-sugar fruits, vegetables, and olive oils [151]. There are also some specific illustrations of particular foods that are helpful. For example, poultry eggs contain a number of bioactive components that can modulate inflammation [154]. There is a suggestion that eating raw eggs can reduce the morbidity of chronic mycoplasmal infections, but this has not been tested in controlled clinical trials. Probiotics are important and will be discussed in another section (6.5).

6.2. Vitamins, Minerals and Proteins

Chronic illness patients, and especially patients with chronic pathogenic mycoplasma infections, should be considered depleted in many essential vitamins and minerals that can be replaced by diet or dietary supplements [132] [151] [152] [153]. For example, we have suggested adding sublingual vitamin B complex (riboflavin, thiamine, niacin, pantothenic acid, pyridoxine, folic acid, biotin, cyanocobalamin, choline), and vitamins A, C and E to routine vitamin use [132] [152]. Other mitochondrial replacement supplements that may be useful include coenzyme Q-10 (CoQ_{10}) L-carnitine, Alpha-lipoic acid, and glycerolphospholipids (see Section 6) [151] [152] [153] [155].

Important minerals that are often depleted during mycoplasmal infections include zinc, magnesium chromium and selenium. These can be replaced by taking mineral supplements [151] [152] [155].

Other useful supplements containing beta-carotene, bioflavoids, and amino acids, such as L-cyseine, L-tyrosine and L-glutamine. A supplement formulation of specific amino acids, Fatigue Reviva™, has been developed to support amino acid depletion found in conditions of sub-health, such as chronic infections [157]. Buhner [153] also suggests adding L-arginine, L-tryptophan, L-threonine, and L-serine to this mixture of amino acids. Poultry eggs were mentioned in section 6.1 as an excellent source of proteins and other nutritional components.

6.3. Modifying Mycoplasma Microenvironments

Pathogenic mycoplasmas can reside in both extracellular and intracellular microenvironments. In the latter they are adept at modifying their microenvironments to optimize uptake or cellular transport of nutrients. Mycoplasmas can also adjust their enzymatic and other activities, depending on where they are located. To provide microenvironments that are not optimal for mycoplasmas, it is imperative that acidity be controlled at both the extracellular and intracellular levels [151]. This has been done extracellularly with antacids or diet that is not acidic. For intracellular regulation of acidity vitamin C, lemon-lime water, a lemon-olive oil drink or fruits and vegetables that raise intracellular pH have been used to modulate intracellular environments to more alkaline states [132] [151].

Next, the oxidative states of tissues and cells need to be under control to prevent excess oxidative stress, but not too much control, because many natural functions require some free radical oxidants for metabolic and gene regulation. This can safely be accomplished with dietary use of particular anti-oxidants, and there are some excellent examples of natural anti-oxidants that can be useful. Some are mentioned in section 6.2 and elsewhere [132] [151] [152] [153]. Since normal cellular signaling and metabolism are dependent on the presence of natural cellular oxidants, it is important to not interfere with normal cellular oxidative balance, but at the same time controlling excess oxidative stress [158]. Dietary antioxidants, such as beta-cryptoxanthin found in fruits and vegetables, ap-

pear to be useful in controlling cellular redox states. This turns out to be important in essentially all types of infections, but it has also been found to be particularly important in cancers, neurodegenerative and other diseases [159].

A very safe and effective method to control oxidative stress and reduce inflammation during mycoplasma treatment is the use of hydrogenized water [160]. Molecular hydrogen reduces oxidative stress and cytotoxic oxygen radicals by a gene regulation mechanism not by direct interactions [161]. Hydrogenized water does not interfere with conventional or integrative therapies against mycoplasmas. It can be easily consumed orally without need to worry about contraindications [160].

As mentioned in Section 5, chronic infections can hide in biofilms and be refractory to anti-microbial treatments. There are several natural biofilm-degrading enzymes that can be useful during anti-microbial treatments, such as nattokinase, lumbrokinase, and serrapeptase [151]. Together with monolauren or glycerol monolaurate, an anti-biofilm agent derived from lauric acid found in coconut and other oils [162], these are useful in disrupting biofilm and potentially improving therapy against a variety of microbial pathogens that hide in biofilms. A natural sweetener, stevia, extracted from the leaves of *Stevia rebaudiana* plant, has also been shown to be a natural biofilm disrupter [163]. Other natural herbal biofilm disrupters, such as extracts of pomegranate, maple syrup, cinnamon and peppermint, have been used separately or together to inhibit biofilms [151].

Chronic infections also stimulate the release of inflammatory cytokines, as discussed in section 5. Natural cytokine inhibitors have been used to reduce inflammation, such as alpha-lipoic acid (ALA). As mentioned previously, ALA has been used for mitochondrial support [155] [156]. ALA has also been shown to be a good inhibitor of inflammatory cytokines in rheumatoid arthritis patients [164]. Other herbs and vegetables, such as curcumin, broccoli seed extracts, cordyceps, Chinese skullcap, Isatis and Houttuynia extracts, have also been used to reduce specifically inflammation during mycoplasmal infections [151] [153].

Inside cells mycoplasmas compete with mitochondria for metabolites and precursor molecules [153] [155] [156]. In most cells mitochondria supply approximately 90% of cellular energy needs, so having mycoplasmas competing for precursor molecules and stealing high-energy mitochondrial products can result in reduced mitochondrial function and output. This translates to loss of overall energy that is perceived as fatigue and reduced function [155] [156]. Thus it is important that these depleted components be replaced, such as L-carnitine, ALA, CoQ_{10}, and other components, especially membrane glycerolphospholipids [155] [156] (This will be discussed separately in Section 6.8).

6.4. Herbal Use to Reduce Jarisch-Herxheimer Reactions

In Section 5, I discussed the J-H adverse reactions that are often found during treatment of pathogenic mycoplasma infections. Various natural supplements and herbs have been used to minimize J-H reactions. Horowitz [151] has discussed the use of smilix, redroot, and boneset to reduce J-H reactions. He has

also discussed the employment of *Stephania* root, *Andrographis*, and polygonum (a form of resveratrol) for J-H reactions [151]. In addition, Cordyceps, Chinese skullcap, Isatis and Houttuynia extracts, and the flavones baicalein and wagonin as well as extracts of *Scutellaria baicalensis* and *Acacia catechu* have been utilized by either Horowitz [151] or Buchner [153] to reduce inflammation and decrease J-H reactions.

6.5. Probiotics and Prebiotics for Gut Health

It is becoming increasing clear that maintenance of an appropriate balance of gut microbes is essential to gastrointestinal and general health [165] [166]. Probiotics and prebiotics (growth and other factors that support gut microbiome balance) have been used in a variety of clinical conditions to rebalance gut microbe proportions and reduce pathogenic bacterial strains. There are a few examples, such as *Clostridium difficile*-associated diarrhea, where this has this resulted in significant changes in disease status or morbidity [166]. Thus there has been considerable interest in using probiotics and prebiotics in clinical practice, especially for prevention of antibiotic-associated diarrhea [167].

In pathogenic mycoplasma-infected individuals undergoing antibiotic therapy it is especially important to maintain gut microbial balance, so addition of probiotic/prebiotic supplements would be expected to useful for patients [132] [151] [152]. We generally suggest that supplementation be done at maximal suggested doses of probiotic mixtures 1 - 2 hours after oral antibiotics have been taken to reduce gut microbiome susceptibility to unabsorbed antibiotics [132]. Certain probiotic mixtures can lower inflammation and abdominal symptoms, and these appear to be useful [151]. Addition of fiber to the diet, such as flaxseed, has also been suggested to increase bowel movement in order to remove toxic substances and allergins [132] [151].

The use of oral digestive enzymes to help dissolve biofilm was discussed in section 6.3. Such enzymes have also been used to improve food uptake and maintenance of a healthy gut microbiome.

6.6. Other Herbals and Natural Remedies

In Section 5, the use of conventional therapies, for the most part antibiotics, to treat pathogenic mycoplasma infections was discussed. This should remain an important, documented strategy for suppressing mycoplasmas. However, some patients do not tolerate favorably antibiotics, and often discontinue their use at inappropriate times. Also, after antibiotic therapies are discontinued, patients often require some additional anti-microbial tactics to maintain mycoplasmal suppression [132]. Therefore, the use of herbal supplements has been added to mycoplasma treatment strategies during and after antibiotics have been stopped [132] [151] [152] [153]. Although for the most part these herbs and natural remedies have not been tested in controlled clinical trials with mycoplasma-infected patients, their use has become so widespread that they should be

considered on a patient-to-patient basis.

There are many types of herbal extract mixtures and formulations that have been used in patients with pathogenic mycoplasma infections [153]. These include those that contain, in no particular order, cordeceps (*Cordycepsspp.*), Chinese skullcap (*Scutellaria sinensis*), Chinese senega root (*Polygala tenufolia*), kudzu root (*Pueraria lobata*), isatis (*Isatis indigotica* and other species), houttuynia (*Houttuynia cordata*) olive leaf (*Olea europaea*), berberine (*Berberis spp.*), uva ursi (*Arctostaphylos uva-ursi*), pomegranate (*Punica granatum*), anogeissus (*Anogeissus leiocarpus*), tea tree (*Melaleuca alternifolia*), noni (*Morinda citrifolia*), among others. Buhner [153] has carefully discussed the available evidence justifying the use of these herbal formulations in patients with mycoplasmal infections. Although some herbal mixtures are available as commercial products, such as Myco+ (by *Rain Tree* available on Amazon.com), for the most part the anti-mycoplasma herbal mixtures can be found on various websites [132] or must be developed de novo for individual patients [153].

6.7. Oxidative and Other Therapies

Pathogenic mycoplasmas can exist in both aerobic and anaerobic environments, but in certain tissues, such as joint synovia with lower oxygen tension, they can be considered as borderline anaerobes [1]. As a borderline anaerobe, they should be susceptible to suppression by high oxygen tension. Thus one approach to mycoplasma therapy has been to increase the overall oxygen content of tissues by hyperbaric oxygen, hydrogen peroxide or ozone. These approaches to mycoplasma therapy appear to be mainly cytostatic not cytotoxic, but they have been used in conjunction with other therapies to treat mycoplasma-infected individuals (discussed in [132]).

Immunotherapy has been suggested for mycoplasmal infections, but there are few examples in the literature. In non-human primates mycoplasmal infections have been prophylactically treated by prior immunization with immunogenic surface components [118] [168]. Humans have also been immunized with mycoplasma (*M. pneumoniae*) components, and the results have generally been promising [169] [170]. Further efforts will have to be made in this area before effective vaccines are available, including the development of more sensitive and quantitative techniques of measurement of mycoplasmas in cells and body fluids [170].

Immune modulators have been used to boost host immunity. For the most part these have been non-specific modulators [132] [133]. For example, bioactive whey proteins, polysaccharides, mushroom extracts, transfer factors and other natural products have been used in patients with mycoplasmal infections. In general, their clinical effectiveness has not been carefully evaluated, and their efficacy has not been documented in clinical trials.

One problem that can potentially interfere with anti-mycoplasmal therapies is the presence of heavy metals in tissues and organs, which can contribute to in-

flammation and immune dysfunction [121] [151]. Horowitz has discussed the use of chelation and nutritional supplementation in the removal of heavy metals from patients undergoing treatments for chronic infections [151].

6.8. Membrane Lipid Replacement

Membrane Lipid Replacement (MLR) is the critically important oral supplementation of membrane glycerolphospholipids to provide replacement molecules that are damaged or lost during pathogenic mycoplasma infections [171] [172] [173]. Replacement membrane phospholipids are important for a variety of cellular and tissue functions and for general health [172]. Patients with chronic illnesses and infections as well as aged individuals are often deficient in undamaged membrane glycerolphospholipids, because dietary sources usually cannot provide enough undamaged MLR lipids for maintenance of cellular membranes [172] [173].

The use of oral MLR glycerolphospholipids with unsaturated fatty acids (example, NTFactor Lipids) in doses ranging from 3 - 4 g per day has proven to be safe and effective for many clinical conditions, including acute and chronic mycoplasmal infections (reviewed in [172] [174]). MLR results in the actual replacement of damaged membrane phospholipids with undamaged (unoxidized) phospholipids to ensure proper functioning of cellular and intracellular membranes, such as mitochondrial inner membranes essential to cellular energy production.

MLR has also been used to reduce fatigue, pain, gastrointestinal and other symptoms [172] [173] [174], and in mycoplasma-infected and other patients it can restore critical cellular membrane functions [173]. MLR can also help remove hydrophobic neurotoxins, such as mold toxins, that can interfere with cellular response mechanisms and mitochondrial function [171] [172].

7. Final Comments

There is now a growing awareness that many chronic illnesses are associated with pathogenic mycoplasma infections that are either responsible (causative) for the illness or more likely acting as cofactors, progression factors and certainly factors which are responsible for aggravating patient morbidity, and in some cases possibly even contributing to fatal outcomes [7] [45] [46] [47]. In addition, pathogenic mycoplasmas routinely occur with other pathogens, often in complex multi-infectious syndromes [121]. Once they have been diagnosed, and that is often difficult with currently available tools, the successful treatment of pathogenic mycoplasmas has been challenging, especially in their chronic forms where the reduced effectiveness of conventional therapies, usually antibiotics, has forced us to consider other treatment options, some of which are discussed in this review.

The present review lists a number of contributing factors for successful suppression of pathogenic mycoplasma infections and extends previous efforts [132]

[133]. However, each of these and other treatment suggestions is ever changing and often difficult to apply to individual cases in a uniform regimen. General recommendations are to initially apply conventional therapies that employ antibiotics best suited for treatment of specific *Mycoplasma* species at maximum suggested doses either daily or every other day, unless patients are unable to tolerate antibiotics. In this case, integrative therapies should be considered as frontline therapy. The extended treatments of mycoplasmal infections require patience and the flexibility to change treatment strategies if patients do not slowly show improvements in symptom severities. Various integrative medicines and patient support strategies should also be employed during initial and follow-on therapies as outlined but not limited to this review [121] [151]. As stated here and elsewhere, a major problem is under-treatment, and practitioners will have to decide how long to treat individual patients with mycoplasmal and other infections. This author is also acutely aware of the inability of most treatments to completely eliminate chronic pathogenic mycoplasmas. Thus we have focused on suppression, support and recovery rather than complete eradication of pathogenic mycoplasmas, because the latter may be extremely difficult if not impossible to achieve.

Many of the suggestions presented here do not strictly depend on the properties of the pathogen(s) itself; they are instead focused on modification of host microenvironments. Thus different approaches to the therapy of complex infectious illnesses and syndromes involving pathogenic mycoplasmas should always be considered. For example, the MSIDS multifactorial treatment model proposed by Horowitz [121] identifies and treats overlapping symptoms and their downstream effects rather than focusing on individual infections. This and other approaches to therapy may be necessary to overcome the complex natures of pathogenic mycoplasmas and their treatments, including other co-infections, and the abilities of pathogenic mycoplasmas to persist and cause multiple, overlapping symptoms.

Acknowledgements

I would like to thank present and former colleagues for their intellectual support, and especially my late spouse, Dr. Nancy Nicolson (1953-2019), who survived lethal mycoplasmal infections and stood by me for so many years. This contribution was supported by internal funds from the Department of Molecular Pathology, The Institute for Molecular Medicine.

Disclosures

The author is a part-time consultant to Nutritional Therapeutics, Inc., Allergy Research Group, Inc., Naturally plus USA and UNIVA Naturally plus Taiwan.

Conflicts of Interest

The author has no conflicts of interest to declare regarding this contribution.

References

[1] Razin, S, Yogev, D. and Naot, Y. (1998) Molecular Biology and Pathogenicity of Mycoplasmas. *Microbiology and Molecular Biology Reviews*, **62**, 1094-1156. https://mmbr.asm.org/content/62/4/1094

[2] Narita, M. (2016) Classification of Extrapulmonary Manifestations Due to *Mycoplasma pnaumoniae* Infection on the Basis of Possible Pathogenesis. *Frontiers in Microbiology*, **7**, Article 23. https://doi.org/10.3389/fmicb.2016.00023

[3] Taylor-Robinson, D. and Jensen, J.S. (2011) *Mycoplasma genitalium*: From Chrysalis to Multicolored Butterfly. *Clinical Microbiology Reviews*, **24**, 498-514. https://cmr.asm.org/content/24/3/498 https://doi.org/10.1128/CMR.00006-11

[4] Atkinson, T.P. (2018) Mollicutes: *Mycoplasma pneumoniae*. In: Ragab, G., Atkinson, T., Prescott, S. and Matthew, L., Eds., *The Microbiome in Rheumatic Diseases and Infection*, Springer International Publishing, Berlin, 103-111. https://doi.org/10.1007/978-3-319-79026-8_10

[5] Baseman, J.B. and Tully, J.G. (1997) Mycoplasmas: Sophisticated, Reemerging, and Burdened by Their Notoriety. *Emerging Infectious Diseases*, **3**, 21-32. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2627593/pdf/9126441.pdf https://doi.org/10.3201/eid0301.970103

[6] Nicolson, G.L., Nasralla, M.Y., Haier, J., Erwin, R., Nicolson, N.L. and Ngwenya, R. (1999) Mycoplasmal Infections in Chronic Illnesses: Fibromyalgia and Chronic Fatigue Syndromes, Gulf War Illness, HIV-AIDS and Rheumatoid Arthritis. *Medical Sentinel*, **4**, 172-176. https://haciendapublishing.com/medicalsentinel/mycoplasmal-infections-chronic-illnesses-fibromyalgia-and-chronic-fatigue-syndromes-

[7] Nicolson, G.L., Nasralla, M.Y. and Nicolson, N.L. (1999) The Pathogenesis and Treatment of Mycoplasmal Infections. *Antimicrobial and Infectious Disease Newsletter*, **17**, 81-87. http://lymeaware.free.fr/lyme/Publications/The%20Pathogenesis%20and%20Treatment%20of%20Mycoplasmal%20Infections.pdf https://doi.org/10.1016/S1069-417X(00)88885-8

[8] Agnas, B. (1997) Sexual Transmitted Diseases (*Mycoplasma homonis*, *Ureaplasma urealyticum* and *Chlamydia trachomatis*) among Young Females. *Orvosi Hetilap* (*Hungarian Medical Journal*), **138**, 799-803. https://akademiai.com/doi/pdf/10.1556/650.1997.03.02

[9] Svenstrup, H.F., Fedder, J., Kristoffersen, S.E., Trolle, B., Birkelund, S. and Christiansen, G. (2008) *Mycoplasma genitalium*, *Chlamydia trachomatis* and Tubal Factor Infertility—A Prospective Study. *Fertility and Sterility*, **90**, 513-520. https://www.fertstert.org/article/S0015-0282(07)00107-0/fulltext https://doi.org/10.1016/j.fertnstert.2006.12.056

[10] Murtha, A.P. and Edwards, J.M. (2014) The Role of *Mycoplasma* and *Ureaplasma* in Adverse Pregnancy Outcomes. *Obstetrics and Gynecology Clinics of North America*, **41**, 615-627. https://doi.org/10.1016/j.ogc.2014.08.010 https://www.obgyn.theclinics.com/article/S0889-8545(14)00072-2/pdf

[11] Bitnun, A. and Richardson, S.E. (2010) *Mycoplasma pneumoniae*: Innocent Bystander or a True Cause of Central Nervous System Disease? *Current Infectious Disease Report*, **12**, 282-290. https://link.springer.com/article/10.1007%2Fs11908-010-0105-4 https://doi.org/10.1007/s11908-010-0105-4

[12] Weidenfeld, J., Wohlman, A. and Gallily, R. (1995) *Mycoplasma fermentans* Activates the Hypothalamo-Pituitary Adrenal Axis in the Rat. *NeuroReport*, **6**, 910-912. https://journals.lww.com/neuroreport/Abstract/1995/04190/Mycoplasma_fermentans_activates_the.21.aspx https://doi.org/10.1097/00001756-199504190-00021

[13] Wohlman, A., Gallily, R., Yirmiya, R. and Weidenfeld, J. (1997) Characterization of the Effect of *Mycoplasma fermentans* on the Hypothalamo-Pituitary-Adrenal Axis. *Neuroendocrinology*, **66**, 221-228. https://doi.org/10.1159/000127241

[14] Fadiel, A., Eichenbaum, K.D., El Semary, N. and Epperson, B. (2007) Mycoplasma Genomics: Tailoring the Genome for Minimal Life Requirements through Reductive Evolution. *Frontiers in Bioscience*, **12**, 2020-2028. https://www.bioscience.org/2007/v12/af/2207/list.htm

[15] Razin, S. (1985) Molecular Biology and Genetics of Mycoplasmas (Mollicutes). *Microbiological Reviews*, **49**, 419-455. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC373046/

[16] Razin, S. (1997) Comparative Genomics of Mycoplasmas. *Weiner Klinischer Wochenschrift*, **109**, 551-556. https://www.ncbi.nlm.nih.gov/pubmed/9286058

[17] Glass, J.I., Assad-Garcia, N., Alperovich, N., Yooseph, S., Lewis, M.R., Maruf, M., Hutchison, C.A., Smith, H.O. and Venter, J.C. (2006) Essential Genes of a Minimal Bacterium. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 425-430. https://www.pnas.org/content/pnas/103/2/425.full.pdf https://doi.org/10.1073/pnas.0510013103

[18] Reddy, S.P., Rasmussen, W.G. and Baseman, J.B. (1996) Isolation and Characterization of Transposon Tn4001-Generated, Cytadherence-Deficient Transformants of *Mycoplasma pneumoniae* and *Mycoplasma genitalium*. *FEMS Immunology and Medical Microbiology*, **15**, 199-211. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1574-695X.1996.tb00086.x https://doi.org/10.1111/j.1574-695X.1996.tb00086.x

[19] Baseman, J.B., Reddy, S.P. and Dallo, S.F. (1996) Interplay between Mycoplasma Surface Proteins, Airway Cells, and the Protean Manifestations of Mycoplasma-Mediated Human Infections. *American Journal of Respiratory and Critical Care Medicine*, **154**, S137-S144. https://www.atsjournals.org/doi/abs/10.1164/ajrccm/154.4_Pt_2.S137 https://doi.org/10.1164/ajrccm/154.4_Pt_2.S137

[20] Schaeverbeke, T., Vernhes, J.P., Lequen, L., Bannwarth, B., Bebear, C. and Dehais, J. (1997) Mycoplasmas and Arthritides. *Reviews in Rheumatology English Education*, **64**, 120-128. https://www.ncbi.nlm.nih.gov/pubmed/9085447

[21] Rottem, S. (2003) Interaction of Mycoplasmas with Host Cells. *Physiological Reviews*, **83**, 417-432. https://www.physiology.org/doi/pdf/10.1152/physrev.00030.2002 https://doi.org/10.1152/physrev.00030.2002

[22] Klement, M.L., Öjemyr, L., Tagscherer, K.E., Widmalm, G. and Wieslander, A. (2007) A Processive Lipid Glycosyltransferase in the Small Human Pathogen *Mycoplasma pneumoniae:* Involvement in Host Immune Response. *Molecular Microbiology*, **65**, 1444-1457. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1365-2958.2007.05865.x https://doi.org/10.1111/j.1365-2958.2007.05865.x

[23] Zhang, Q. and Wise, K.S. (1996) Molecular Basis of Size and Antigenic Variation of a *Mycoplasma hominis* Adhesin Encoded by Divergent *vaa* Genes. *Infection and Immunity*, **64**, 2737-2744. https://iai.asm.org/content/iai/64/7/2737.full.pdf

[24] McGowin, C.L. and Totten, P.A. (2017) The Unique Microbiology and Molecular Pathogenesis of *Mycoplasma genitalium*. *Journal of Infectious Diseases*, **216**, S382-S388. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5853509/ https://doi.org/10.1093/infdis/jix172

[25] Burgos, R., Pich, O.Q., Ferrer-Navorro, M., Baseman, J.B., Querol, E. and Pinol, J. (2006) *Mycoplasma genitalium* P140 and P110 Cytadhesions Are Reciprocally Stabilized and Required for Cell Adhesion and Terminal-Organelle Development. *Journal of Bacteriology*, **188**, 8627-8637. https://jb.asm.org/content/188/24/8627 https://doi.org/10.1128/JB.00978-06

[26] Svenstrup, H.F., Jensen, J.S., Gevaert, K., Birkelund, S. and Christiansen, G. (2006) Identification and Characterization of Immunogenic Proteins of *Mycoplasma genitalium*. *Clinical and Vaccine Immunology*, **13**, 913-922. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1539121/ https://doi.org/10.1128/CVI.00048-06

[27] Christodoulides, A., Gupta, N., Yacoubian, V., Maithel, N., Parker, J. and Kelesides, T. (2018) The Role of Lipoproteins in Mycoplasma-Mediated Immunomodulation. *Frontiers in Microbiology*, **9**, Article 1682. https://www.frontiersin.org/articles/10.3389/fmicb.2018.01682/full https://doi.org/10.3389/fmicb.2018.01682

[28] Zhang, Y., Mei, S., Zhou, Y., Huang, M., Dong, G. and Chen, Z. (2016) Cytokines as the Good Predictors of Refractory *Mycoplasma pneumoniae* Pneumonia in School-Aged Children. *Scientific Reports*, **6**, Article No. 37037. https://www.nature.com/articles/srep37037 https://doi.org/10.1038/srep37037

[29] Mühlradt, P.F., Kiess, M., Meyer, H., Süssmuth, R. and Jung, G. (1997) Insolation, Structure Elucidation, and Synthesis of a Macrophage Stimulatory Lipoprotein from *Mycoplasma fermentans* Acting at Picomolar Concentration. *Journal of Experimental Medicine*, **185**, 1951-1958. http://jem.rupress.org/content/185/11/1951/tab-pdf https://doi.org/10.1084/jem.185.11.1951

[30] Mühlradt, P.F., Meyer, H. and Jansen, R. (1996) Identification of S-(2,3-Dihydroxypropyl)Cystein in a Macrophage-Activating Lipopeptide from *Mycoplasma fermentans*. *Biochemistry*, **35**, 7781-7786. https://pubs.acs.org/doi/pdf/10.1021/bi9602831 https://doi.org/10.1021/bi9602831

[31] Frisch, M., Gradehandt, G. and Muhlradt, P.F. (1996) *Mycoplasma fermentans*-Derived Lipid Inhibits Class II Major Histocompatibility Complex Expression without Mediation by Interleukin-6, Interleukin-10, Tumor Necrosis Factor, Transforming Growth Factor-Beta, Type I Interferon, Prostaglandins or Nitric Oxide. *European Journal of Immunology*, **26**, 1050-1057. https://onlinelibrary.wiley.com/doi/10.1002/eji.1830260514 https://doi.org/10.1002/eji.1830260514

[32] Sasaki, Y., Blanchard, A., Watson, H.L., Garcia, S., Dulioust, A., Montagnier, L. and Gougeon, M.L. (1995) *In Vitro* Influence of *Mycoplasma penetrans* on Activation of Peripheral T Lymphocytes from Healthy Donors or Human Immunodeficiency Virus-Infected Individuals. *Infection and Immunity*, **63**, 4277-4283. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC173607/pdf/634277.pdf

[33] Kaufmann, A., Mühlradt, P.F., Gemsa, D. and Sprenger, H. (1999) Induction of Cytokines and Chemokines in Human Monocytes by *Mycoplasma fermentans*-Derived Lipoprotein MALP-2. *Infection and Immunity*, **67**, 6303-6308.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC97033/

[34] Brenner, C., Wroblewski, H., Henaff, M.L., Montagnier, L. and Blanchard, A. (1997) Spiralin, a Mycoplasmal Membrane Protein, Induces T-Cell-Independent B-Cell Blastogenesis and Secretion of Proinflammatory Cytokines. *Infection and Immunity*, **65**, 4322-4329. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC175619/pdf/654322.pdf

[35] He, J., Liu, M., Ye, Z., Tan, T., Liu, X., You, X., Zeng, Y. and Wu, Y. (2016) Insights into the Pathogenesis of *Mycoplasma penumoniae*. *Molecular Medicine Reports*, **14**, 4030-4036. https://www.spandidos-publications.com/mmr/14/5/4030 https://doi.org/10.3892/mmr.2016.5765

[36] Bendjennat, M., Blanchard, A., Loutfi, M., Montagnier, L. and Bahraoui, E. (1999) Role of *Mycoplasma penetrans* Endonuclease P40 as a Potential Pathogenic Determinant. *Infection and Immunity*, **67**, 4456-4462. https://iai.asm.org/content/67/9/4456

[37] Minion, F.C., Jarvill-Taylor, K.J., Billings, D.E. and Tigges, E. (1993) Membrane-Associated Nuclease Activities in Mycoplasmas. *Journal of Bacteriology*, **175**, 7842-7847. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC206960/ https://doi.org/10.1128/jb.175.24.7842-7847.1993

[38] Saitoh, S., Wada, T., Narita, M., Kohsaka, S., Mizukami, S., Togashi, T. and Kajii, N. (1993) *Mycoplasma pneumoniae* Infection May Cause Striatal Lesions Leading to Acute Neurologic Dysfunction. *Neurology*, **43**, 2150-2151. https://doi.org/10.1212/WNL.43.10.2150

[39] Rawadi, G., Roman-Roman, S., Castedo, M., Dutilleul, V., Susin, S., Marchetti, P., Geuskens, M. and Kroemer, G. (1996) Effects of *Mycoplasma fermentans* on the Myelomonocytic Linage. Different Molecular Endities with Cytokine-Inducing and Cytocidal Potential. *Journal of Immunology*, **156**, 670-678. https://www.jimmunol.org/content/156/2/670

[40] Waites, K.B., Xiao, L., Liu, Y., Balish, M.F. and Atkinson, T.P. (2017) *Mycoplasma pneumonia* from the Respiratory Tract and beyond. *Clinical Microbiology Reviews*, **30**, 747-809. https://doi.org/10.1128/CMR.00114-16

[41] Komada, Y., Zhang, X.L., Zhou, Y.W., Ido, M. and Azuma, E. (1997) Apoptotic Cell Death of Human Lymphoblastoid Cells Induced by Arginine Deaminase. *International Journal of Hematology*, **65**, 129-141. https://doi.org/10.1016/S0925-5710(96)00538-5

[42] Molinos, L., Fernandez, R., Dominguez, M.J., Riesgo, C., Escudero, C. and Martinez, J. (1997) Adenosine Deaminase Activity in the Aetiological Diagnosis of Community-Acquired Pneumonia. *Scandinavian Journal of Infectious Disease*, **29**, 287-290. https://www.ncbi.nlm.nih.gov/pubmed/9255891 https://doi.org/10.3109/00365549709019044

[43] Becker, A., Kannan, T.R., Taylor, A.B., Pakhornova, O.N., Zhang, Y., Somarajan, S.R., Galaleldeen, A., Holloway, S.P., Baseman, J.B. and Hart, P.J. (2015) Structure of CARDS Toxin, a Unique ADP-Ribosylating and Vacuolating Cytotoxin from *Mycoplasma pneumoniae*. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 5165-5170. https://doi.org/10.1073/pnas.1420308112

[44] Muir, M.T., Cohn, S.M., Louden, C., Kannan, T.R. and Baseman, J.B. (2011) Novel Toxin Assays Implicate *Mycoplasma pneumoniae* in Prolonged Ventilator Course and Hypoxemia. *Chest*, **139**, 305-310. https://journal.chestnet.org/article/S0012-3692(11)60069-X/pdf https://doi.org/10.1378/chest.10-1222

[45] Nicolson, G.L. (2008) Chronic Infections in Neurodegenerative and Neurobehavioral Diseases. *Laboratory Medicine*, **39**, 291-299. https://academic.oup.com/labmed/article/39/5/291/2504709 https://doi.org/10.1309/96M3BWYP42L11BFU

[46] Nicolson, G.L. and Haier, J. (2009) Role of Chronic Bacterial and Viral Infections in Neurodegenerative, Neurobehavioral, Psychiatric, Autoimmune and Fatiguing Illnesses: Part 1. *British Journal of Medical Practitioners*, **2**, 20-28. https://www.bjmp.org/content/role-chronic-bacterial-and-viral-infections-neurodegenerative-neurobehavioral-psychiatric-au

[47] Nicolson, G.L. and Haier, J. (2010) Role of Chronic Bacterial and Viral Infections in Neurodegenerative, Neurobehavioral, Psychiatric, Autoimmune and Fatiguing Illnesses: Part 2. *British Journal of Medical Practitioners*, **3**, 301-311. https://www.bjmp.org/content/role-chronic-bacterial-and-viral-infections-neurodegenerative-neurobehavioural-psychiatric-a

[48] Nicolson, G.L. and Nicolson, N.L. (1996) Diagnosis and Treatment of Mycoplasmal Infections in Persian Gulf War Illness-CFIDS Patients. *International Journal of Occupational Medicine*, *Immunology and Toxicology*, **5**, 69-78. http://www.immed.org/GWI%20Research%20docs/06.26.12.updates.pdfs.gwi/Nicolson-IJOMIT1996.pdf

[49] Lin, L.-J., Chang, F.-C., Chi, H., Jim, W.-T., Huang, D.T., Kung, Y.-H., Huang, C.-Y., Chiu, N.-C. and Chang, L. (2019) The Diagnostic Value of Serological Studies in Pediatric Patients with Acute *Mycoplasma pneumonia* Infection. *Journal of Microbiology*, *Immunology and Infection*, in press. https://reader.elsevier.com/reader/sd/pii/S1684118218300756?token=71A32FBE2D315829BB4DFFC4A7DDDC107BADBCCFF9715DF60C78017A8DCE47F1346A0DA5365AD0088E1FDC1AAD5D8825 https://doi.org/10.1016/j.jmii.2018.09.001

[50] Sauter, P.M., van Rossum, A.M.C. and Vink, C. (2014) *Mycoplasma pneumoniae* in Children: Carriage, Pathogenesis and Antibiotic Resistance. *Current Opinion in Infectious Diseases*, **27**, 220-227. https://journals.lww.com/co-infectiousdiseases/Abstract/2014/06000/Mycoplasma_pneumoniae_in_children___carriage,%20.3.aspx https://doi.org/10.1097/QCO.0000000000000063

[51] Jacobs, E., Ehrhardt, I. and Dumke, R. (2015) New Insights in the Outbreak Pattern of *Mycoplasma pneumoniae*. *International Journal of Medical Microbiology*, **305**, 705-708. https://www.ncbi.nlm.nih.gov/pubmed/26319941 https://doi.org/10.1016/j.ijmm.2015.08.021

[52] Cimolai, N., Wensley, D., Seear, M. and Thomas, E.T. (1995) *Mycoplasma pneumoniae* as a Cofactor in Severe Respiratory Infections. *Clinical Infectious Diseases*, **21**, 1182-1185. https://academic.oup.com/cid/article-abstract/21/5/1182/357396?redirectedFrom=fulltext https://doi.org/10.1093/clinids/21.5.1182

[53] Gray, G.C., Duffy, L.B., Paver, R.J., Putnam, S.D., Reynolds, R.J. and Cassell, G.H. (1997) *Mycoplasma pneumoniae*: A Frequent Cause of Pneumonia among U.S. Marines in Southern California. *Military Medicine*, **162**, 524-526. https://academic.oup.com/milmed/article/162/8/524/4831693 https://doi.org/10.1093/milmed/162.8.524

[54] Mishra, R., Cano, E., Venkatram, S. and Diaz-Fuentes, G. (2017) An Interesting Case of Mycoplasma Pneumonia Associated Multisystem Involvement and Diffuse

Alveolar Hemorrhage. *Respiratory Medicine Case Reports*, **21**, 78-81.
https://www.sciencedirect.com/science/article/pii/S2213007117300692
https://doi.org/10.1016/j.rmcr.2017.03.022

[55] Jackson, D.J., Gern, J.E. and Lemanske Jr., R.F. (2016) The Contributions of Allergic Sensitization and Respiratory Pathogens to Asthma Inception. *Clinical Reviews in Allergy and Immunology*, **137**, 659-665.
https://www.jacionline.org/article/S0091-6749(16)00106-8/pdf
https://doi.org/10.1016/j.jaci.2016.01.002

[56] Holt, R.D., Wilson, M. and Musa, S. (1995) Mycoplasmas in Plaque and Saliva of Children and Their Relationship to Gingivitis. *Journal of Periodontology*, **66**, 97-101. https://aap.onlinelibrary.wiley.com/doi/10.1902/jop.1995.66.2.97
https://doi.org/10.1902/jop.1995.66.2.97

[57] Kwek, H.S., Wilson, M. and Newman, H.N. (1990) Mycoplasma in Relation to Gingivitis and Periodontitis. *Journal of Clinical Periodontology*, **17**, 119-122.
https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-051X.1990.tb01073.x?sid=nlm%3Apubmed
https://doi.org/10.1111/j.1600-051X.1990.tb01073.x

[58] Borak, J. and Lefkowitz, R.Y. (2016) Bronchial Hyperresponsiveness. *Occupational Medicine*, **66**, 95-105. https://academic.oup.com/occmed/article/66/2/95/2750597
https://doi.org/10.1093/occmed/kqv158

[59] Parrott, G.L., Kinjo, T. and Fujita, J. (2016) A Compendium for *Mycoplasma pneumoniae*. *Frontiers in Microbiology*, **7**, Article 513.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4828434/pdf/fmicb-07-00513.pdf
https://doi.org/10.3389/fmicb.2016.00513

[60] Seggev, J.S., Sedmak, G.V. and Kurup, V. (1996) Isotype-Specific Antibody Responses to Acute *Mycoplasma pneumoniae* Infection. *Annals of Allergy, Asthma and Immunology*, **77**, 67-73.
https://www.annallergy.org/article/S1081-1206(10)63482-5/pdf
https://doi.org/10.1016/S1081-1206(10)63482-5

[61] Combaz-Söhnchen, N. and Kuhn, A. (2017) A Systematic Review of Mycoplasma and Ureaplasma in Urogynaecology. *Geburtschilfe und Frauenheilkunde*, **77**, 1299-1303.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5734936/

[62] Mardh, P.A., Elshibly, S., Kallings, I. and Hellberg, D. (1997) Vaginal Flora Changes Associated with *Mycoplasma hominis*. *American Journal of Obstretrics and Gynecology*, **176**, 173-178. https://www.ajog.org/article/S0002-9378(97)80031-2/fulltext
https://doi.org/10.1016/S0002-9378(97)80031-2

[63] Al-Farraj, D.A. and Moubayed, N.M. (2019) The Association between Sociodemographic, Hormonal, Tubo-Ovanian Factors and Bacterial Count in Chlamydia and Mycoplasma Infections with Infertility. *Saudi Journal of Biological Sciences*, **26**, 20-23. https://www.sciencedirect.com/science/article/pii/S1319562X16301632
https://doi.org/10.1016/j.sjbs.2016.11.006

[64] Waites, K.B., Katz, B. and Schelonka, R.L. (2005) Mycoplasmas and Ureaplasmas as Neonatal Pathogens. *Clinical Microbiology Reviews*, **18**, 757-789.
https://cmr.asm.org/content/18/4/757
https://doi.org/10.1128/CMR.18.4.757-789.2005

[65] Schuppe, H.C., Pilatz, A., Hossain, H., Diemer, T., Wagenlehner, F. and Weidner, W. (2017) Urogenital Infection as a Risk Factor for Male Infertility. *Deutsches Ärzteblatt International*, **114**, 339-346.
https://www.aerzteblatt.de/int/archive/article/188504

https://doi.org/10.3238/arztebl.2017.0339

[66] Cassell, G.H., Waites, K.B., Watson, H.L., Crouse, D.T. and Harasawa, R. (1993) *Ureaplasma urealyticum* Intraurerine Infection: Role in Prematurity and Diseases in Newborns. *Clinical Microbiology Reviews*, **6**, 69-87.
https://cmr.asm.org/content/cmr/6/1/69.full.pdf
https://doi.org/10.1128/CMR.6.1.69

[67] Lemaitre, M., Henin, Y., Destouesse, F., Ferrieux, C., Montagnier, A. and Blanchard, A. (1992) Role of Mycoplasmas in the Cytopathic Effect Induced by Human Immunodeficiency Virus Type 1 in Infected Cell Lines. *Infection and Immunity*, **60**, 742-748.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC257548/pdf/iai00027-0034.pdf

[68] Ainsworth, J.G., Katseni, V., Hourshid, S., Ball, S., Cattell, V. and Taylor-Robinson, D. (1994) *Mycoplasma fermentans* and HIV-Associated Nephropathy. *Journal of Infection*, **29**, 323-326.
https://www.journalofinfection.com/article/S0163-4453(94)91289-0/fulltext
https://doi.org/10.1016/S0163-4453(94)91289-0

[69] Blanchard, A. (1997) Mycoplasmas and HIV Infection, a Possible Interaction through Immune Activation. *Wien Klinische Wochenschrift*, **109**, 590-593.
https://www.ncbi.nlm.nih.gov/pubmed/9286065

[70] Montagnier, L. and Blanchard, A. (1993) Mycoplasmas as Cofactors in Infection Due to the Immunodeficiency Virus. *Clinical Infectious Disease*, **17**, S309-S315.
https://www.jstor.org/stable/4457254?seq=1-%20page_scan_tab_contents#page_scan_tab_contents

[71] Li, J.-L., Matsuda, K., Takagi, M. and Yamamoto, N. (1997) Detection of Antibodies against Phosphocholine-Containing Aminoglycoglycerolipid Specific to *Mycoplasma fermentans* in HIV-1 Infected Individuals. *Journal of Immunological Methods*, **208**, 103-113.
https://www.sciencedirect.com/science/article/pii/S002217599700135X
https://doi.org/10.1016/S0022-1759(97)00135-X

[72] Kocacic, R., Launay, V., Tuppin, P., Lafeuillade, A., Feuillie, V., Montagnier, L. and Grau, O. (1996) Search for the Presence of Six Mycoplasma Species in Peripheral Blood Mononuclear Cells of Subjects Seropositive and Seronegative for Human Immunodeficiency Virus. *Journal of Clinical Microbiology*, **34**, 1808-1810.
https://jcm.asm.org/content/34/7/1808

[73] Sloot, N., Hollandt, H., Gatermann, S. and Dalhoff, K. (1996) Detection of *Mycoplasma* sp. in Bronchoalveolar Lavage of AIDS Patients with Pulmonary Infiltrates. *Zentralblatt für Bakeriologie*, **284**, 75-79.
https://www.sciencedirect.com/science/article/abs/pii/S0934884096801563
https://doi.org/10.1016/S0934-8840(96)80156-3

[74] Bisset, L.R. (1994) Molecular Mimicry in the Pathogenesis of AIDS: The HIV/MHC/Mycoplasma Triangle. *Medical Hypothesis*, **43**, 388-396.
https://www.sciencedirect.com/science/article/abs/pii/0306987794900140
https://doi.org/10.1016/0306-9877(94)90014-0

[75] Jefferies, W.M. (1998) The Etiology of Rheumatoid Arthritis. *Medical Hypotheses*, **51**, 111-114.
https://www.sciencedirect.com/science/article/abs/pii/S0306987798901037
https://doi.org/10.1016/S0306-9877(98)90103-7

[76] Ford, D. (1991) The Microbial Causes of Rheumatoid Arthritis. *Journal of Rheumatology*, **18**, 1441-1442.

[77] Rivera, A., Yáñez, A., León-Tello, G., Gil, C., Barba, E. and Cedillo, L. (2002) Experimental Arthritis Induced by a Clinical *Mycoplasma fermentans* Isolate. *BMC Musculoskeletal Disorders*, **3**, Article No. 15.
https://bmcmusculoskeletdisord.biomedcentral.com/articles/10.1186/1471-2474-3-15
https://doi.org/10.1186/1471-2474-3-15

[78] Schaeverbeke, T., Renaudin, H., Clerc, M., Lequen, L., Vernhes, J.P., De Barbeyrac, B., Bannwarth, B., Bébéar, C. and Dehais, J. (1997) Systemic Detection of Mycoplasmas by Culture and Polymerase Chain Reaction (PCR) Procedures in 209 Synovial Fluid Samples. *British Journal of Rheumatology*, **36**, 310-314.
https://academic.oup.com/rheumatology/article/36/3/310/1782740
https://doi.org/10.1093/rheumatology/36.3.310

[79] Haier, J., Nasralla, M., Franco, A.R. and Nicolson, G.L. (1999) Detection of Mycoplasmal Infections in Blood of Patients with Rheumatoid Arthritis. *Rheumatology*, **38**, 504-509. https://academic.oup.com/rheumatology/article/38/6/504/1783454
https://doi.org/10.1093/rheumatology/38.6.504

[80] Furr, P.M., Taylor-Robinson, D. and Webster, A.D. (1994) Mycoplasmas and Ureaplasmas in Patients with Hypogammaglobulinaemia and Their Role in Arthritis: Microbiological Observations over Twenty Years. *Annuals of Rheumatic Diseases*, **53**, 183-187. https://ard.bmj.com/content/53/3/183
https://doi.org/10.1136/ard.53.3.183

[81] Cole, B.C. and Griffith, M.M. (1993) Triggering and Exacerbation of Autoimmune Arthritis by the *Mycoplasma arthritidis* Superantigen MAM. *Arthritis and Rheumatism*, **36**, 994-1002. https://onlinelibrary.wiley.com/doi/10.1002/art.1780360717
https://doi.org/10.1002/art.1780360717

[82] Kirchhoff, H., Binder, A., Runge, M., Meier, B., Jacobs, R. and Busche, K. (1989) Pathogenic Mechanisms in the *Mycoplasma arthritidis* Polyarthritis of Rats. *Rheumatology International*, **9**, 193-196.
https://link.springer.com/article/10.1007/BF00271879
https://doi.org/10.1007/BF00271879

[83] Narita, M. (2010) Pathogenesis of Extrapulmonary Manifestations of *Mycoplasma pneumoniae* Infection with Special Reference to Pneumonia. *Journal of Infection and Chemotherapy*, **16**, 162-169.
https://www.sciencedirect.com/science/article/abs/pii/S1341321X10705973
https://doi.org/10.1007/s10156-010-0044-X

[84] Feizi, T. and Loveless, R.W. (1996) Carbohydrate Recognition by *Mycoplasma pneumoniae* and Pathologic Consequences. *American Journal of Respiratory and Critical Care Medicine*, **154**, S133-S136.
https://www.atsjournals.org/doi/pdf/10.1164/ajrccm/154.4_Pt_2.S133
https://doi.org/10.1164/ajrccm/154.4_Pt_2.S133

[85] Kaneoka, H. and Naito, S. (1997) Superantigens and Autoimmune Diseases. *Nippon Rinsho. Japanese Journal of Clinical Medicine*, **55**, 1363-1369.
https://europepmc.org/abstract/med/9200919

[86] Mattson, M.P. (2004) Infectious Agents and Age-Related Neurodegenerative Disorders. *Ageing Research Reviews*, **3**, 105-120.
https://www.sciencedirect.com/science/article/pii/S1568163703000394
https://doi.org/10.1016/j.arr.2003.08.005

[87] Zasler, N.D., Martelli, M.F. and Jacobs, H.E. (2013) Neurobehavioral Disorders. *Handbook of Clinical Neurology*, **110**, 377-388.

https://www.ncbi.nlm.nih.gov/pubmed/23312657
https://doi.org/10.1016/B978-0-444-52901-5.00032-0

[88] Walling, A.D. (1999) Amyotrophic Lateral Sclerosis: Lou Gehrig's Disease. *American Family Physician*, **59**, 1489-1496.
https://www.aafp.org/afp/1999/0315/p1489.html

[89] Nicolson, G.L., Nasralla, M., Haier, J. and Promfret, J. (2002) High Frequency of Systemic Mycoplasmal Infections in Gulf War Veterans and Civilians with Amyotrophic Lateral Sclerosis (ALS). *Journal of Clinical Neurosciences*, **9**, 525-529.
https://www.sciencedirect.com/science/article/abs/pii/S0967586801910756
https://doi.org/10.1054/jocn.2001.1075

[90] Gil, C., González, A.A.S., León, I.S., Rivera, A., Olea, R.S. and Cedillo, L. (2014) Detection of Mycoplasmas in Patients with Amyotrophic Lateral Sclerosis. *Advances in Microbiology*, **4**, 712-719.
http://www.scirp.org/journal/paperinformation.aspx?paperid=49483
https://doi.org/10.4236/aim.2014.411077

[91] Libbey, J.E., Cusick, M.F. and Fujinami, R.S. (2013) Role of Pathogens in Multiple Sclerosis. *International Review of Immunology*, **33**, 266-283.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4369909/pdf/nihms672154.pdf
https://doi.org/10.3109/08830185.2013.823422

[92] Bahar, M., Ashtari, F., Aghaei, M., Akbari, M., Salari, M. and Ghalamkari, S. (2012) *Mycoplasma pneumoniae* Seropositvity in Iranian Patients with Relapsing-Remitting Multiple Sclerosis: A Randomized Case-Control Study. *Journal of Pakistan Medical Association*, **62**, S6-S8. https://www.ncbi.nlm.nih.gov/pubmed/?term=22768448

[93] Sriram, S., Mitchell, W. and Stratton, C. (1998) Multiple Sclerosis Associated with *Chlamydia pneumoniae* Infection of the CNS. *Neurology*, **50**, 571-572.
https://n.neurology.org/content/50/2/571
https://doi.org/10.1212/WNL.50.2.571

[94] Tselis, A. (2011) Evidence for Viral Etiology of Multiple Sclerosis. *Seminars in Neurology*, **31**, 307-316.
https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0031-1287656
https://doi.org/10.1055/s-0031-1287656

[95] Nicolson, G.L., Gan, R., Nicolson, N.L. and Haier, J. (2007) Evidence for *Mycoplasma spp.*, *Chlamydia pneumoniae*, and Human Herpes Virus-6 in the Blood of Patients with Autistic Spectrum Disorders. *Journal of Neuroscience Research*, **85**, 1143-1148. https://onlinelibrary.wiley.com/doi/abs/10.1002/jnr.21203
https://doi.org/10.1002/jnr.21203

[96] Bransfield, R.C., Wulfman, J.S., Harvey, W.T. and Usman, A.I. (2008) The Association between Tick-Borne Infections, Lyme Borreliosis and Autism Spectrum Disorders. *Medical Hypotheses*, **70**, 967-974.
https://www.sciencedirect.com/science/article/abs/pii/S0306987707005786?via=ihub
https://doi.org/10.1016/j.mehy.2007.09.006

[97] Kuhn, M., Grave, S., Bransfield, R. and Harris, S. (2012) Long Term Antibiotic Therapy May Be an Effective Treatment for Children Co-Morbid with Lyme Disease and Autism Spectrum Disorder. *Medical Hypotheses*, **78**, 606-615.
https://www.sciencedirect.com/science/article/abs/pii/S0306987712000485
https://doi.org/10.1016/j.mehy.2012.01.037

[98] Fukuda, K., Strauss, S.E., Hickie, I., Sharpe, M.C., Dobbins, J.G. and Komaroff, A.

(1994) The Chronic Fatigue Syndrome: A Comprehensive Approach to Its Definition and Study. *Annals of Internal Medicine*, **121**, 953-959.
https://annals.org/aim/article-abstract/708271/chronic-fatigue-syndrome-comprehensive-approach-its-definition-study
https://doi.org/10.7326/0003-4819-121-12-199412150-00009

[99] Carruthers, B.M., Jain, A.K., De Meirleir, K.L., Peterson, D.L., Klimas, N.G. and Lerner, A.M. (2003) Malgic Encephalomyelitis/Chronic Fatigue Syndrome. Clinical Working Case Definition, Diagnostic and Treatment Protocols. *Journal of Chronic Fatigue Syndrome*, **11**, 7-115.
https://www.tandfonline.com/doi/abs/10.1300/J092v11n01_02
https://doi.org/10.1300/J092v11n01_02

[100] Nicolson, G.L., Nasralla, M., De Meirleir, K., Gan, R. and Haier, J. (2003) Evidence for Bacterial (*Mycoplasma*, *Chlamydia*) and Viral (HHV-6) Co-Infections in Chronic Fatigue Syndrome Patients. *Journal of Chronic Fatigue Syndrome*, **11**, 7-19. https://www.tandfonline.com/doi/abs/10.1300/J092v11n02_02
https://doi.org/10.1300/J092v11n02_02

[101] Nasralla, M., Haier, J. and Nicolson, G.L. (1999) Multiple Mycoplasmal Infections Detected in Blood of Patients with Chronic Fatigue Syndrome and/or Fibromyalgia. *European Journal of Microbiology and Infectious Diseases*, **18**, 859-865.
https://link.springer.com/article/10.1007/s100960050420
https://doi.org/10.1007/s100960050420

[102] Nijs, J., Nicolson, G.L., De Becker, P., Coomans, D. and De Meirleir, K. (2006) High Prevalence of *Mycoplasma* Infections among European Chronic Fatigue Syndrome Patients. Examination of Found *Mycoplasma* Species in Blood of Chronic Fatigue Syndrome Patients. *FEMS Immunology and Medical Microbiology,* **34**, 209-214.
https://onlinelibrary.wiley.com/doi/10.1111/j.1574-695X.2002.tb00626.x
https://doi.org/10.1111/j.1574-695X.2002.tb00626.x

[103] Nicolson, G.L., Gan, R. and Haier, J. (2003) Multiple Co-Infections (*Mycoplasma*, *Chlamydia*, Human Herpes Virus-6) in Blood of Chronic Fatigue Syndrome Patients: Association with Signs and Symptoms. *Acta Pathologica Microbiologica Immunologica Scandanavia* (*APMIS*), **111**, 557-566.
https://onlinelibrary.wiley.com/doi/10.1034/j.1600-0463.2003.1110504.x
https://doi.org/10.1034/j.1600-0463.2003.1110504.x

[104] Breeding, P.C., Russell, N.C. and Nicolson, G.L. (2012) Integrative Model of Chronically Activated Immune-Hormonal Pathways Important in the Generation of Fibromyalgia. *British Journal of Medical Practitioners*, **5**, a524.
https://www.bjmp.org/content/integrative-model-chronically-activated-immune-hormonal-pathways-important-generation-fibrom

[105] Nicolson, G.L., Nicolson, N.L., Berns, P., Nasralla, M.Y., Haier, J. and Nass, M. (2003) Gulf War Illnesses: Chemical, Biological and Radiological Exposures Resulting in Chronic Fatiguing Illnesses Can Be Identified and Treated. *Journal of Chronic Fatigue Syndrome*, **11**, 135-154.
https://www.tandfonline.com/doi/abs/10.1300/J092v11n01_04
https://doi.org/10.1300/J092v11n01_04

[106] Janulewicz, P., Krengel, M., Quinn, E., Heeren, T., Toomey, R., Killiany, R., Zundel, C., Ajama, J., O'Callaghan, J., Steele, L., Klimas, N. and Sullivan, K. (2018) The Multiple Hit Hypothesis for Gulf War Illness: Self-Reported Chemical/Biological Weapons Exposure and Mild Traumatic Brain Injury. *Brain Sciences*, **8**, Article 198.
https://www.mdpi.com/2076-3425/8/11/198
https://doi.org/10.3390/brainsci8110198

[107] Bach, J.F. (2005) Infections and Autoimmune Diseases. *Journal of Autoimmunity*, **25**, 74-80. https://www.sciencedirect.com/science/article/pii/S0896841105001320 https://doi.org/10.1016/j.jaut.2005.09.024

[108] Gorthi, S.P., Kapoor, L., Chaudhry, R., Sharma, N., Perez-Perez, G.I., Panigrahi, P. and Behari, M. (2006) Guillain-Barré Syndrome: Association with *Campylobacter jujuniand*, *Mycoplasma pneumoniae* Infections in India. *National Medicine Journal India*, **19**, 137-139. http://archive.nmji.in/archives/Volume_19_3_May_June2006/short_report/Guillain_Barre_syndrome.htm

[109] Shulman, S.T. and Stanford, T. (2009) Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococci (PANDAS): Update. *Current Opinion in Pediatrics*, **21**, 127-130. https://journals.lww.com/co-pediatrics/Abstract/2009/02000/Pediatric_autoimmune_neuropsychiatric_disorders.18.aspx https://doi.org/10.1097/MOP.0b013e32831db2c4

[110] Müller, N., Riedel, M., Blendinger, C., Oberle, K., Jacobs, E. and Abele-Horn, M. (2004) *Mycoplasma pneumoniae* Infection and Tourette's Syndrome. *Psychiatry Research*, **129**, 119-125. https://www.sciencedirect.com/science/article/abs/pii/S0165178104002082 https://doi.org/10.1016/j.psychres.2004.04.009

[111] Krause, D.L. and Müller, N. (2012) The Relationship between Tourette's Syndrome and Infections. *Open Neurology Journal*, **6**, 124-128. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3514747/ https://doi.org/10.2174/1874205X01206010124

[112] Ainsworth, J.G.E., Esterbrook, P.J., Clarke, J., Gilroy, C.B. and Taylor-Robinson, D. (2001) An Association of Disseminated *Mycoplasma fermentans* in HIV-1 Positive Patients with Non-Hodgkin's Lymphoma. *International Journal of STD and AIDS*, **12**, 499-504. https://journals.sagepub.com/doi/abs/10.1258/0956462011923589 https://doi.org/10.1258/0956462011923589

[113] Henrich, B., Rumming, M., Sczyrba, A., Velleuer, E., Dietrich, R., Gerlach, W., Gombert, M., Rahn, S., Stoye, J., Borkhardt, A. and Fischer U. (2014) *Mycoplasma salivarium* as a Dominant Colonizer of Fanconi Anaemia Associated Oral Carcinoma. *PLoS ONE*, **9**, e92297. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3958540/ https://doi.org/10.1371/journal.pone.0092297

[114] Barykova, Y.A., Logunov, D.Y., Shmarov, M.M., Vinarov, A.Z., Fiev, D.N., Vinarova, N.A., Rakovskaya, I.V., *et al.* (2011) Association of *Mycoplasma hominis* Infection with Prostate Cancer. *Oncotargets*, **2**, 289-297. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3248169/pdf/oncotarget-02-289.pdf https://doi.org/10.18632/oncotarget.256

[115] Jiang, S., Zhang, S., Langenfeld, J., Lo, S.-C. and Rogers, M.B. (2008) Mycoplasma Infection Transforms Normal Lung Cells and Induces Bone Morphogenetic Protein 2 Expression by Post-Transcriptional Mechanisms. *Journal of Cellular Biochemistry*, **104**, 580-594. https://onlinelibrary.wiley.com/doi/abs/10.1002/jcb.21647 https://doi.org/10.1002/jcb.21647

[116] Zhang, S., Tsai, S. and Lo, S.-C. (2006) Alteration of Gene Expression Profiles during Mycoplasma-Induced Malignant Cell Transformation. *BMC Cancer*, **6**, Article No. 116. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1559712/pdf/1471-2407-6-116.pdf

https://doi.org/10.1186/1471-2407-6-116

[117] Zella, D., Curreli, S., Benedetti, F., Krishnan, S., Cocchi, F., Latinovic, O.S., Denaro, F., Romerio, F., *et al.* (2018) Mycoplasma Promotes Malignant Transformation *in Vivo*, and Its DnaK, a Bacterial Chaperone Protein, Has Broad Oncogenic Properties. *Proceedings of the National Academy of Sciences of the United States of America*, **115**, E12005-E12014. https://www.pnas.org/content/115/51/E12005
https://doi.org/10.1073/pnas.1815660115

[118] Matsuda, K. (2015) A Novel Therapeutic Strategy for Mycoplasma Infectious Disease. *Personalized Medicine Universe*, **4**, 32-39.
https://www.sciencedirect.com/science/article/abs/pii/S2186495015000164?via=ihub
https://doi.org/10.1016/j.pmu.2015.04.005

[119] Cameron, D.J., Johnson, L.B. and Maloney, E.L. (2014) Evidence Assessments and Guideline Recommendations in Lyme Disease: The Clinical Management of Known Tick Bites, Erythema Migrans Rashes and Persistent Disease. *Expert Reviews of Anti-Infective Therapy*, **12**, 1103-1135.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4196523/pdf/ERZ-12-1103.pdf
https://doi.org/10.1586/14787210.2014.940900

[120] Horowitz, R.I. and Freeman, P.R. (2019) Precision Medicine: Retrospective Chart Review and Data Analysis of 200 Patients on Dapsone Combination Therapy for Chronic Lyme Disease/Post-Treatment Lyme Disease Syndrome: Part 1. *International Journal of General Medicine*, **12**, 101-119.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6388746/pdf/ijgm-12-101.pdf
https://doi.org/10.2147/IJGM.S193608

[121] Horowitz, R.I. and Freeman, P.R. (2018) Precision Medicine: The Role of MSIDS Model in Defining, Diagnosing and Treating Chronic Lyme Disease/Post Treatment Lyme Disease Syndrome and Other Chronic Illnesses: Part 2. *Healthcare* (*Basel*), **6**, Article 129.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6316761/pdf/healthcare-06-00129.pdf
https://doi.org/10.3390/healthcare6040129

[122] Nicolson, G.L., Nicolson, N.L. and Haier, J. (2007) Chronic Fatigue Syndrome Patients Subsequently Diagnosed with Lyme Disease *Borrelia burgdorferi*: Evidence for *Mycoplasma* Species Co-Infections. *Journal of Chronic Fatigue Syndrome*, **14**, 5-17. https://www.tandfonline.com/doi/abs/10.3109/10573320802091809
https://doi.org/10.1080/10573320802091809

[123] Nicolson, G.L. (2017) Mycoplasma and Other Intracellular Bacterial Infections in Rheumatic Diseases: Comorbid Condition or Cause? *Open Journal of Tropical Medicine*, **1**, 016-017. https://www.peertechz.com/articles/OJTM-1-103.php
https://doi.org/10.17352/ojtm.000003

[124] Biondi, E., McCulloh, R., Alverson, B., Klein, A., Dixon, A. and Ralston, S. (2014) Treatment of *Mycoplasma pneumoniae*: A Systematic Review. *Pediatrics*, **133**, 1081-1090. https://pediatrics.aappublications.org/content/133/6/1081.short
https://doi.org/10.1542/peds.2013-3729

[125] Kenny, G.E. and Cartwright, F.D. (2001) Susceptibilities of *Mycoplasma hominis*, *M. pneumoniae*, and *Ureaplasma urealyticum* to GAR-936, Dalfopristin, Dirithromycin, Evernimicin, Gatifloxacin, Linezolid, Moxifloxacin, Quinupristin-Dalfopristin, and Telithromycin Compared to Their Susceptibilities to Reference Macrolides, Tetracyclines, and Quinolones. *Antimicrobial Agents and Chemotherapy*, **45**, 2604-2608. https://aac.asm.org/content/45/9/2604

https://doi.org/10.1128/AAC.45.9.2604-2608.2001

[126] Arai, S., Gohara, Y., Kuwano, K. and Kawashima, T. (1992) Antimycoplasmal Activities of New Quinolones, Tetracyclines and Macrolides against *Mycoplasma pneumoniae*. *Antimicrobial Agents and Chemotherapy*, **36**, 1322-1324.
https://aac.asm.org/content/36/6/1322
https://doi.org/10.1128/AAC.36.6.1322

[127] Hannan, P.C.T. (1998) Comparative Susceptibilities of Various AIDS-Associated and Human Genital Tract Mycoplasmas and Strains of *Mycoplasma pneumoniae* to 10 Classes of Antimicrobial Agents *in Vitro*. *Journal of Medical Microbiology*, **47**, 1115-1122.
https://www.microbiologyresearch.org/docserver/fulltext/jmm/47/12/medmicro-47-12-1115.pdf?expires=1565590277&id=id&accname=guest&checksum=737B6C7EF2B638462C05CD2B646414B7
https://doi.org/10.1099/00222615-47-12-1115

[128] Renaudin, H. and Bébéar, C. (1990) Comparative *in Vitro* Activity of Azithromycin, Clarithromycin, Erythromycin and Lomefloxacin against *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. *European Journal of Clinical Microbiology and Infectious Diseases*, **9**, 838-841.
https://link.springer.com/article/10.1007/BF01967388
https://doi.org/10.1007/BF01967388

[129] Roberts, M.C., Koutsky, L.A., Holmes, K.K., LeBlanc, D.L. and Kenny, G.E. (1985) Tetracycline-Resistant *Mycoplasma hominis* Strains Contain Streptococcal *tetM* Sequences. *Antimicrobial Agents and Chemotherapy*, **28**, 141-143.
https://aac.asm.org/content/28/1/141
https://doi.org/10.1128/AAC.28.1.141

[130] Roberts, M.C. and Kenny, G.E. (1986) Dissemination of the *tetM* Tetracycline Resistance Determinant to *Ureaplasma urealyticum*. *Antimicrobial Agents and Chemotherapy*, **29**, 350-352. https://aac.asm.org/content/29/2/350
https://doi.org/10.1128/AAC.29.2.350

[131] Bébéar, C.M., Renaudin, J., Charron, A., Renaudin, H., de Barbeyrac, B., Schaeverbeke, T. and Bébéar, C. (1999) Mutations in the *gyrA*, *parC* and *parE* Genes Associated with Fluoroquinolone Resistance in Clinical Isolates of *Mycoplasma hominis*. *Antimicrobial Agents and Chemotherapy*, **43**, 954-956.
https://aac.asm.org/content/43/4/954
https://doi.org/10.1128/AAC.43.4.954

[132] Nicolson, G.L. (1998) Some Considerations When Undergoing Treatment for Chronic Illnesses and Autoimmune Diseases, plus Supplemental Suggestions. *International Journal of Medicine*, **1**, 123-128.
http://www.immed.org/treatment%20considerations/03.12.2012update/IMM.updates%2023.Sept.2018/Treatment%20Considerations%202018.pdf

[133] Nicolson, G.L., Nasralla, M.Y., Franco, A.R., Nicolson, N.L., Erwin, R., Ngwenya, R. and Berns, P.A. (2000) Diagnosis and Integrative Treatment of Intracellular Bacterial Infections in Chronic Fatigue and Fibromyalgia Syndromes, Gulf War Illness, Rheumatoid Arthritis and Other Chronic Illnesses. *Clinical Practice of Alternative Medicine*, **1**, 92-102.
http://www.immed.org/Fatigue%20Illness/06.08.12%20pdfs/CPAM-GLNetal.-00.1.21RTF.pdf

[134] D'Alonzo, R., Mencaroni, E., Di Genova, L., Laino, D., Principi, N. and Esposito, S. (2018) Pathogenesis and Treatment of Neurological Diseases Associated with *Mycoplasma pneumoniae* Infection. *Frontiers in Microbiology*, **9**, Article 2751.

https://www.frontiersin.org/articles/10.3389/fmicb.2018.02751/full
https://doi.org/10.3389/fmicb.2018.02751

[135] Tilley, B.C., Alarcon, G.S., Heyse, S.P., Trentham, D.E., Neuner, R., Kaplan, D.A., Clegg, D.O., Leisen, J.C.C., Buckley, L., Cooper, S.M., Duncan, H., Pillemer, S.R., Tuttleman, M. and Fowler, S.E. (1995) Minocycline in Rheumatoid Arthritis: A 48-week, Double-Blind, Placebo-Controlled Trial. *Annals of Internal Medicine*, **122**, 81-89. https://doi.org/10.7326/0003-4819-122-2-199501150-00001
https://annals.org/aim/article-abstract/708353/minocycline-rheumatoid-arthritis-48-week-double-blind-placebo-controlled-trial

[136] Suzuki, S., Yamazaki, T., Narita, M., Okazaki, N., Suzuki, I., Andoh, T., Matsuoka, M., Kenri, T., Arakawa, Y. and Sasaki, T. (2006) Clinical Evaluation of Macrolide-Resistant *Mycoplasma pneumoniae*. *Antimicrobial Agents and Chemotherapy*, **50**, 709-712. https://aac.asm.org/content/50/2/709
https://doi.org/10.1128/AAC.50.2.709-712.2006

[137] Todd, S.R., Dahlgren, F.S., Traeger, M.S., Beltrán-Aguilar, E.D., Marianos, D.W., Hamilton, C., McQuiston, J.H. and Regan, J.J. (2015) No Visible Dental Staining in Children Treated with Doxycycline for Suspected Rocky Mountain Spotted Fever. *Journal of Pediatrics*, **166**, 1246-1251.
https://www.jpeds.com/article/S0022-3476(15)00135-3/fulltext
https://doi.org/10.1016/j.jpeds.2015.02.015

[138] Wang, M., Wang, Y., Yan, Y., Zhu, C., Huang, L., Shao, X., Xu, J., Zhu, H., Sun, X., Ji, W. and Chen, Z. (2014) Clinical and Laboratory Profiles of Refractory *Mycoplasma pneumoniae* Pneumonia in Children. *International Journal of Infectious Diseases*, **29**, 18-23.
https://www.ijidonline.com/article/S1201-9712(14)01605-1/fulltext
https://doi.org/10.1016/j.ijid.2014.07.020

[139] Bradshaw, C.R., Jensen, J.S. and Waites, K.B. (2017) New Horizons in *Mycoplasma genitalium* Treatment. *Journal of Infectious Diseases*, **216**, S412-S419.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5853296/pdf/jix132.pdf
https://doi.org/10.1093/infdis/jix132

[140] Chopra, I. and Roberts, M. (2001) Tetracycline Antibiotics: Mode of Action, Applications, Molecular Biology and Epidemiology of Bacterial Resistance. *Microbiology and Molecular Biology Reviews*, **65**, 232-260.
https://mmbr.asm.org/content/65/2/232
https://doi.org/10.1128/MMBR.65.2.232-260.2001

[141] Butler, T. (2017) The Jarish-Herxheimer Reaction after Antibiotic Treatment of Spirochetal Infections: A Review of Recent Cases and Our Understanding of Pathogenesis. *The American Journal of Tropical Medicine and Hygiene*, **96**, 46-52.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5239707/
https://doi.org/10.4269/ajtmh.16-0434

[142] Wormser, G.P., Dattwyler, R.J., Shapiro, E.D., Halperin, J.J., Steere, A.C., Klempner, M.S., Krause, P.J., Bakken, J.S., Strie, F., Stanek, G., Bockenstedt, L., Fish, D., Dumler, J.S. and Nadelman, R.B. (2006) The Clinical Assessment, Treatment and Prevention of Lyme Disease, Human Granulocytic Anaplasmosis and Babesiosis: Clinical Practice Guidelines by the Infectious Disease Society of America. *Clinical Infectious Diseases*, **43**, 1089-1134.
https://academic.oup.com/cid/article/43/9/1089/422463
https://doi.org/10.1086/508667

[143] Lebeaux, D., Ghigo, J.-M. and Belion, C. (2014) Biofilm-Related Infections: Bridging the Gap between Clinical Management and Fundamental Aspects of Recalcitrance

toward Antibiotics. *Microbiology and Molecular Biology Reviews*, **78**, 510-543. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4187679/ https://doi.org/10.1128/MMBR.00013-14

[144] Lewis, K. (2006) Persister Cells, Dormancy and Infectious Disease. *Nature Reviews Microbiology*, **5**, 48-56. https://www.nature.com/articles/nrmicro1557 https://doi.org/10.1038/nrmicro1557

[145] Fox, R.I. (1993) Mechanism of Action of Hydroxychloroquine as an Antirheumatic Drug. *Seminars in Arthritis and Rheumatism*, **32**, 82-91. https://www.sciencedirect.com/science/article/abs/pii/S0049017210800125 https://doi.org/10.1016/S0049-0172(10)80012-5

[146] Lee, K.-Y., Lee, H.-S., Jong, J.-H., Lee, M.-H., Lee, J-S., Burgner, D. and Lee, B.-C. (2006) Role of Prednisolone Treatment in Severe *Mycoplasma pneumoniae* Pneumonia in Children. *Pediatric Pulmonology*, **41**, 263-268. https://onlinelibrary.wiley.com/doi/abs/10.1002/ppul.20374 https://doi.org/10.1002/ppul.20374

[147] Hughes, R.A., Swan, A.V. and van Doorn, P.A. (2014) Intravenous Immunoglobulins for Guillain-Barré Syndrome. *Cochrane Database of Systematic Reviews*, **2014**, Article CD002063. https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD002063.pub6/full?highlightAbstract=syndrom|guillain|withdrawn|barr|barre|syndrome https://doi.org/10.1002/14651858.CD002063.pub6

[148] Chevert, S., Hughes, R.A. and Annane, D. (2017) Plasma Exchange for Guillain-Barré Syndrome. *Cochrane Database of Systematic Reviews*, **2017**, Article CD001798. https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD001798.pub3/full?highlightAbstract=withdrawn|plasm|exchang|plasma https://doi.org/10.1002/14651858.CD001798.pub3

[149] Yrjänheikki, J., Tikka, T., Keinänen, R., Goldsteins, G., Chan, P.H. and Koistinaho, J. (1999) A Tetracycline Derivative, Minocycline, Reduces Inflammation and Protects against Focal Cerebral Ischemia with a Wide Therapeutic Window. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 13496-13500. https://www.pnas.org/content/pnas/96/23/13496.full.pdf https://doi.org/10.1073/pnas.96.23.13496

[150] Tamaoki, J., Kadota, J. and Takizawa, H. (2004) Clinical Implications of the Immunomodulatory Effects of Macrolides. *The American Journal of Medicine Supplements*, **117**, 5-11. https://www.sciencedirect.com/science/article/abs/pii/S154827660400024X https://doi.org/10.1016/j.amjmed.2004.07.023

[151] Horowitz, R.I. (2017) How Can I Get Better? An Action Plan for Treating Resistant Lyme and Chronic Disease. St. Martin's Press, New York. https://us.macmillan.com/books/9781250070548

[152] Nicolson, G.L. and Ngwenya, R. (2001) Dietary Considerations for Patients with Chronic Illnesses and Multiple Chronic Infections. A Brief Outline of Eighteen Dietary Steps to Better Health. *Townsend Letter*, **219**, 63-65. http://www.immed.org/treatment%20considerations/08.16.2012update/TownsendDietConsid.-01.7.pdf

[153] Buhner, S.H. (2013) Healing Lyme Disease Coinfections. Complementary and Holistic Treatments for Bartonella and Mycoplasma. Healing Arts Press, Rochester. https://www.simonandschuster.com/books/Healing-Lyme-Disease-Coinfections/Stephen-Harrod-Buhner/9781620550083

[154] Anderson, C.J. (2015) Bioactive Eggs Components and Inflammation. *Nutrients*, **7**, 7889-7913. https://www.mdpi.com/2072-6643/7/9/5372
https://doi.org/10.3390/nu7095372

[155] Nicolson, G.L. (2014) Mitochondrial Dysfunction and Chronic Disease: Treatment with Natural Supplements. *Alternative Therapies in Health and Medicine*, **20**, 18-25.
http://www.immed.org/treatment%20considerations/05.23.14.Treatment%20Considerations/Mito_Dysfunct_Treatm-NicolsonATHD2014.pdf

[156] Nicolson, G.L. (2014) Mitochondrial Dysfunction and Chronic Disease: Treatment with Natural Supplements. *Integrative Medicine*, **13**, 35-43.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4566449/

[157] Dunstan, R.H., Sparkes, D.L., Roberts, T.K., Crompton, M.J., Gottfries, J. and Dascombe, B.J. (2013) Development of a Complex Amino Acid Supplement, Fatigue Reviva™, or Oral Ingestion: Initial Evaluations of a Product Concept and Impact on Symptoms of Sub-Health in a Group of Males. *Nutrition Journal*, **12**, Article No. 115.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3751078/pdf/1475-2891-12-115.pdf
https://doi.org/10.1186/1475-2891-12-115

[158] Schmidt, H.H.W., Stocker, R., Vollbracht, C., Paulsen, G., Riley, D., Daiber, A. and Cuadrado, A. (2015) Antioxidants in Translational Medicine. *Antioxidants and Redox Signaling*, **23**, 1130-1143.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4657516/pdf/ars.2015.6393.pdf
https://doi.org/10.1089/ars.2015.6393

[159] Burri, B.J., La Frano, M.R. and Zhu, C. (2016) Absorption, Metabolism and Functions of Beta-Cryptoxanthin. *Nutrition Reviews*, **74**, 69-82.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4892306/pdf/nuv064.pdf
https://doi.org/10.1093/nutrit/nuv064

[160] Nicolson, G.L., Ferreira de Mattos, G., Settineri, R., Costa, C., Ellithorpe, R., Rosenblatt, S., La Valle, J., Jimenez, A. and Ohta, S. (2016) Clinical Effects of Hydrogen Administration: From Animal and Human Diseases to Exercise Medicine. *International Journal of Clinical Medicine*, **7**, 32-76.
https://doi.org/10.4236/ijcm.2016.71005

[161] Ohsawa, I., Ishikawa, M., Takahashi, K., Watanabe, M., Nishimaki, K., Yamagata, K., Katsura, K., Katayama, Y., Asoh, S. and Ohta, S. (2007) Hydrogen Acts as a Therapeutic Antioxidant by Selectively Reducing Cytotoxic Oxygen Radicals. *Nature Medicine*, **13**, 688-694. https://doi.org/10.1038/nm1577

[162] Kaufmann, G.F. and Schlievert, P.M. (2015) Non-Aqueous Glycerol Monolaurte Get Exhibits Antibacterial and Anti-Biofilm Activity against Gram-Positive and Gram-Negative Pathogens. *PLoS ONE*, **10**, e120280.
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4370562/pdf/pone.0120280.pdf
https://doi.org/10.1371/journal.pone.0120280

[163] Theophilus, P.A.S., Victoria, M.J., Socarras, K.M., Filush, K.R., Gupta, K., Luecke, D.F. and Sapi, E. (2015) Effectiveness of *Stevia rebaudiana* Whole Leaf Extract against the Various Morphological Forms of *Borrelia burgforferi in Vitro*. *European Journal of Microbiology and Immunology*, **5**, 268-280.
https://akademiai.com/doi/abs/10.1556/1886.2015.00031
https://doi.org/10.1556/1886.2015.00031

[164] Mirtaheri, E., Gargari, B.P., Kolahi, S., Dehghan, P., Asghari-Jafarabadi, M., Hajali-

lou, M., Novin, Z.S. and Abbasi, M.M. (2015) Effects of Alpha-Lipoic Acid Supplementation on Inflammatory Biomarkers and Matrix Metalloproteinase-3 in Rheumatoid Arthritis Patients. *Journal of the American College of Nutrition*, **34**, 310-317. https://www.tandfonline.com/doi/abs/10.1080/07315724.2014.910740 https://doi.org/10.1080/07315724.2014.910740

[165] Gareau, M.G., Sherman, P.M. and Walker, W.A. (2010) Probiotics and the Gut Microbiota in Intestinal Health and Disease. *Nature Reviews of Gastroenterology and Hepatology*, **7**, 503-514. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4748966/pdf/nihms338723.pdf https://doi.org/10.1038/nrgastro.2010.117

[166] Rondanelli, M., Faliva, M.A., Perna, S., Giacosa, A., Peroni, G. and Castellazzi, A.M. (2017) Using Probiotics in Clinical Practice: Where Are We Now? A Review of Existing Meta-Analyses. *Gut Microbes*, **8**, 521-543. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5730384/pdf/kgmi-08-06-1345414.pdf https://doi.org/10.1080/19490976.2017.1345414

[167] Surawicz, C.M. (2008) Role of Probiotics in Antibiotic-Associated Diarrhea, *Clostridium difficile*-Associated Diarrhea, and Recurrent *Clostridium difficile*-Associated Diarrhea. *Journal of Clinical Gastroenterology*, **42**, S64-S70. https://journals.lww.com/jcge/Abstract/2008/07001/Role_of_Probiotics_in_Antibiotic_associated.5.aspx https://doi.org/10.1097/MCG.0b013e3181646d09

[168] Franzoso, G., Hu, P.C., Meloni, G.A. and Barile, M.F. (1994) Immunoblot Analyses of Chimpanzee Sera after Infection and after Immunization and Challenge with *Mycoplasma pneumoniae*. *Infection and Immunity*, **62**, 1008-1014. https://iai.asm.org/content/62/3/1008

[169] Smith, C.B., Friedewald, W.T. and Chanock, R.M. (1967) Inactivated *Mycoplasma pneumoniae* Vaccine. Evaluation in Volunteers. *JAMA*, **199**, 353-358. https://jamanetwork.com/journals/jama/article-abstract/663300 https://doi.org/10.1001/jama.1967.03120060051007

[170] Brown, R.C., Hendley, J.O. and Gwaltney Jr., J.M. (1972) *Mycoplasma pneumonaie* Vaccine: Antigenicity or Fubbered Antigens in Volunteers. *Infection and Immunity*, **5**, 657-661. https://iai.asm.org/content/5/5/657

[171] Nicolson, G.L., Rosenblatt, S., Ferreira de Mattos, G., Settineri, R., Breeding, P.C., Ellithorpe, R.R. and Ash, M.E. (2016) Clinical Uses of Membrane Lipid Replacement Supplements in Restoring Membrane Function and Reducing Fatigue in Chronic Diseases and Cancer. *Discoveries*, **4**, e54. https://www.discoveriesjournals.org/discoveries/D.2016.01.PA-Dr Nicolson.pdf https://doi.org/10.15190/d.2016.1

[172] Nicolson, G.L. and Ash, M.E. (2017) Membrane Lipid Replacement for Chronic Illnesses, Aging and Cancer Using Oral Glycerolphospholipid Formulations with Fructooligosaccharides to Restore Phospholipid Function in Cellular Membranes, Organelles, Cells and Tissues. *Biochimica et Biophysica Acta*, **1859**, 1704-1724. https://doi.org/10.1016/j.bbamem.2017.04.013

[173] Nicolson, G.L. (2016) Membrane Lipid Replacement: Clinical Studies Using a Natural Medicine Approach to Restoring Membrane Function and Improving Health. *International Journal of Clinical Medicine*, **7**, 133-143. http://www.scirp.org/Journal/PaperInformation.aspx?PaperID=63602 https://doi.org/10.4236/ijcm.2016.72015

[174] Nicolson, G.L., Settineri, R., Ferreira, G. and Breeding, P. (2018) Reduction of Pain, Fatigue, Gastrointestinal and Other Symptoms and Improvement in Quality of Life Indicators in Fibromyalgia Patients with Membrane Lipid Replacement Glycerol-phospholipids and Controlled-Release Caffeine. *International Journal of Clinical Medicine*, **9**, 560-579.
https://www.scirp.org/Journal/PaperInformation.aspx?PaperID=86236
https://doi.org/10.4236/ijcm.2018.97051

International Journal of Clinical Medicine, 2019, 10, 523-530
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Ghost Haemoglobin Affecting the Efficacy of Phototherapy

Azizullah Langah[1], Sara Sadiq[2*], Ali Akbar Siyal[1]

[1]Department of Pediatric Medicine, Peoples University of Health Sciences, Nawabshah, Pakistan
[2]Department of Physiology, CMH Institute of Medical Sciences, Bahawalpur, Pakistan
Email: dr.sarabhatti@gmail.com

How to cite this paper: Langah, A., Sadiq, S. and Siyal, A.A. (2019) Ghost Haemoglobin Affecting the Efficacy of Phototherapy. *International Journal of Clinical Medicine*, **10**, 523-530.
https://doi.org/10.4236/ijcm.2019.1010042

Received: August 26, 2019
Accepted: October 12, 2019
Published: October 15, 2019

Abstract

Introduction: Phototherapy is the treatment of choice for neonatal hyperbilirubinemia. It converts the unconjugated bilirubin from polar and neurotoxic Z-Z-bilirubin to the more polar photobilirubin. It has been hypothesized that high level of hemoglobin or hematocrit interferes in the effectiveness of phototherapy. The objective of the current study is to find out the association of hemoglobin/hematocrit to change in total serum bilirubin concentration during phototherapy. Methods: A prospective cohort study was conducted on 296 neonates with uncomplicated hyperbilirubinemia. Before initiating the phototherapy, hemoglobin, hematocrit and total serum bilirubin levels were measured. After treating the neonates with phototherapy using LED-light for 24 hours, the total serum bilirubin level was measured again. The data were analyzed by using SPSS version-20. Results: The majority of study participants were male (60.8%), with mean postnatal age of 4.66 ± 1.65, having mean birth weight of 2.41 ± 0.41. Considering Pearson correlation, the hemoglobin had a significant inverse association with $\Delta TsB_{0\text{-}24}$ while birth weight and postnatal age also had inverse association but p-values were non-significant, while TsB_0 had presented a very weak but significant positive association. The results might be evident of the fact that increase in hemoglobin concentration results in minimal change in total serum bilirubin concentration during phototherapy. Conclusion: Current study found a significant effect of hemoglobin on efficacy of phototherapy, as the change in total serum bilirubin concentration is inversely correlated with the level of hemoglobin. This is important for the physician in treating neonates with hyperbilirubinemia by using phototherapy.

Keywords

Hyperbilirubinemia, Neonatal Jaundice, Phototherapy

1. Introduction

Neonatal Jaundice is not an uncommon condition, approximately 2% - 6% of affected neonates require treatment [1]. Over the past few decades, phototherapy is the treatment of choice for neonatal hyperbilirubinemia, beside intravenous immune globulin (IVIG) and exchange transfusion. Phototherapy prevents the bilirubin to touch the extremely high levels in turn, preventing kernicterus (encephalopathy due to high levels of bilirubin) [2] [3].

The mechanism of action of phototherapy is that it converts the unconjugated bilirubin from polar and neurotoxic Z-Z-bilirubin to the more polar form known as photobilirubin. This transformed photobilirubin consists of configurational Z-E and E-Z-bilirubin isomers and structural E-Z and E-E-lumirubin isomers [3] [4] [5]. These transformed isomers are easy to excrete in both bile without undergoing conjugation and in urine as well [6]. The fact that the mechanism of phototherapy induced transformation occurs predominantly either intravascularly or extravascularly, is under a great debate but the majority favors the idea of intravascular phototherapy induced transformation [7].

In vitro number of studies reported that hemoglobin competes the bilirubin for absorbing light during phototherapy [7] [8] [9] as the erythrocytes do not have nuclei or other cellular organelles so act as the main light absorber [9] [10]. It has been hypothesized that high level of hemoglobin or hematocrit interfered in the effectiveness of phototherapy and only one of the studies confirmed it *in vivo* [1]. It is very important to confirm this hypothesis in our clinical setting so that one can understand the underlying basic mechanism going-on in phototherapy and to suggest a treatment strategy in neonatal hyperbilirubinemia. The objective of the current study is to find out the association of hemoglobin/hematocrit to change in total serum bilirubin concentration during phototherapy. The hypothesis is that the increase in hemoglobin concentration reduces the efficacy of phototherapy.

2. Methods

A prospective cohort study was done from August 2018 up to March 2019 in the neonatal intensive care unit of Peoples University of medical and health sciences (PUMHS) Nawabshah, Pakistan. OpenEpi calculator was used for calculating the sample size which was 306 while the sample population was selected using randomization. The inclusion criteria followed were: 1) healthy term or late preterm (more than 34 weeks of gestation) newborn having hyperbilirubinemia 2) no signs for hemolytic disease 3) clinical signs of jaundice 4) birth weight must be more than 1800 g 5) postnatal age should be more than 24 h but less than 7 days and 6) those who were not previously exposed to phototherapy. Those neonates were excluded from the study whose parents refuse to give the consent or who were either candidate for exchange transfusion or need double phototherapy or got Rhesus (Rh) isoimmunization or having a very high level or rapidly increasing level of total serum bilirubin concentration or patients diagnosed as a septic

case. The basic demographic data including gestational age, postnatal age, gender, birth weight and type of feeding during phototherapy were recorded.

Measurements:

Before initiating the phototherapy, hemoglobin and hematocrit were measured. A trained phlebotomist collected blood samples from the vein/capillary by heel stick method. All the samples were transported under refrigerated temperature and centrifuged within 4-hours of collection. The hemoglobin and hematocrit levels were analyzed by using fully auto hematology analyzer (Model: HL-2400 Plus) in pathology laboratory of PUMHS Nawabshah.

Total serum bilirubin level was measured at the start (TsB_0) and after giving phototherapy for 24 hours (TsB_{24}) in the capillary blood, drawn simultaneously with blood for hemoglobin/hematocrit. It was analyzed by using clinical chemistry analyzer (VitalabSelectra E-series) in pathology laboratory of PUMHS Nawabshah.

Phototherapy:

The instrument used for phototherapy was Infant phototherapy lamp (Model: XHZ-90L, Ningbo David Medical Device CO.LTD), having blue LED-light of 430 - 490 nm spectrum. Before initiating the trial, device was calibrated from the service provider company. Light irradiance was measured by using a radiometer before initiating the trial and during the phototherapy after every eight hours i.e. three times within 24 hours. It was measured at the head, abdomen and knees of the exposed infants and the mean values were calculated.

The distance between the mattress and the light source of phototherapy lamp was about 30 cm and it was adjusted by using a wood template. The average distance from the lamp to the skin of the infant was 22.5 cm. Phototherapy was given to all infants continuously for 24 hours except in between thirty minutes of feeding and nursing care after every three hours. All the participants were naked except the area of diapers and eye pads. Phototherapy was avoided if direct hyperbilirubinemia is more than 20% of TsB_0. Very few of the study participants were excluded from the study because of developing complications like skin rash (erythematous macular rash/purpuric rash), loose stool, overheating and dehydration.

Ethics:

The study got approval from the ethical review committee of PUMHS. Written informed consent was taken from the parents/guardian of the infants before collecting the blood samples.

Statistical Analysis:

The data were analyzed by using Statistical Package for Social Sciences (SPSS) version-20. Mean with standard deviation and range was calculated for numerical data while frequency and percentages for qualitative data. Hemoglobin, hematocrit, TsB_0, TsB_{24}, and change in total serum bilirubin after 24 hours of phototherapy ($\Delta TsB_{0\text{-}24}$) are presented as mean values with range. The linear regression model and Pearson correlation coefficient of $\Delta TsB_{0\text{-}24}$ with hemoglobin, hematocrit, TsB_0, postnatal age and birth weight were calculated. The scatter plot

was drawn to report the distribution of data and association of hemoglobin with $\Delta TsB_{0\text{-}24}$. P-value less than 0.05 was considered as statistically significant.

3. Result

A total of about 306 participants were included in the study but after excluding, those neonates whose parents refuse to give consent or having unsatisfactory results or developed complications, about 296 participants were finally analyzed. Majority of study participants were male (60.8%), with mean postnatal age of 4.66 ± 1.65, having mean birth weight of 2.41 ± 0.41. Looking over the gestational age, majority of the participants were born during 37th week of their gestation as presented in Figure 1. Among the participants, very few were on breast-fed during phototherapy and the mean light irradiance measured was 71.8 ± 11.45. The basic characteristics including demographic variables and irradiance data of study participants are mentioned in Table 1.

At the time of diagnosis, the mean TsB_0 was 16.2 ± 6.92 while mean values of hemoglobin and hematocrit were 16.59 ± 2.75 and 45.78 ± 6.18 respectively. After giving phototherapy for 24 hours, the mean TsB_{24} was 13.15 ± 3.76, so the $\Delta TsB_{0\text{-}24}$ concentration during 24 hours of phototherapy was 5.21 ± 3.05 as mentioned in Table 2.

The linear regression model and Pearson correlation coefficient are calculated in Table 3. The regression analysis showed significant impact of hemoglobin, hematocrit, TsB_0 on $\Delta TsB_{0\text{-}24}$ while non-significant impact of postnatal age and birth weight on $\Delta TsB_{0\text{-}24}$. Considering Pearson correlation, the hemoglobin had significant inverse association with $\Delta TsB_{0\text{-}24}$ while birth weight and postnatal age also had inverse association but p-values were non-significant, while hematocrit and TsB_0 had presented a very weak positive association. The distribution of data in scatter plot with regression line is presented in Figure 2. It displayed a very weak negative correlation which might be evident of the fact that increases in hemoglobin concentration resulting in minimal change in total serum bilirubin concentration during phototherapy.

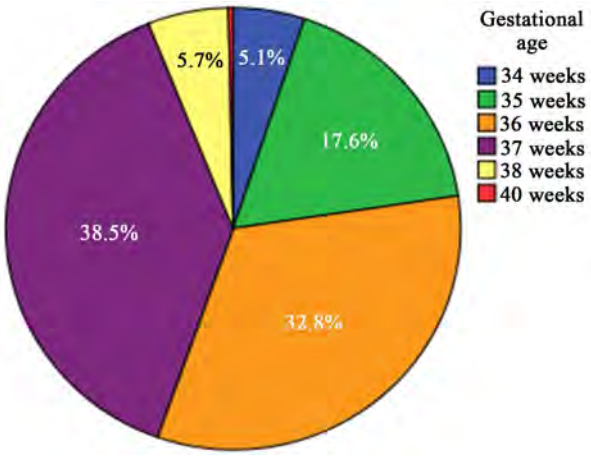


Figure 1. Gestational age of the study participants.

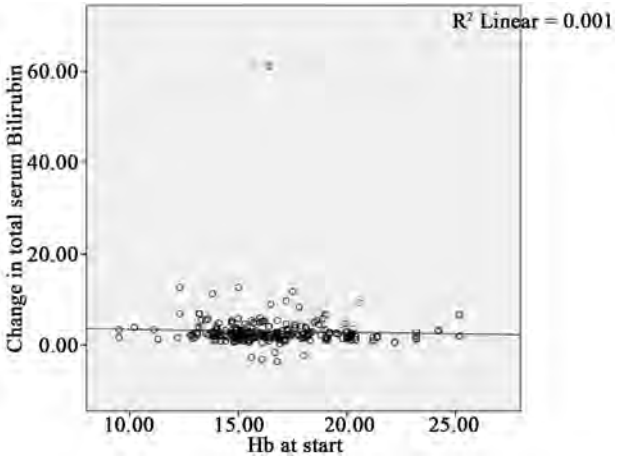


Figure 2. Scatter plot of change in total serum bilirubin during 24 hours of phototherapy versus hemoglobin concentration.

Table 1. Basic characteristics of study participants.

Qualitative Variables		
	n = 296	**%**
Gender		
Male	180	60.8
Female	116	39.2
Type of feeding during phototherapy		
Exclusive breast-fed	81	27.4
Exclusive formula-fed	131	44.3
Mixed breast-fed/ formula-fed	84	28.4
Quantitative Variables		
	Mean ± SD	**Range**
Postnatal age (days)	4.66 ± 1.65	1.36 - 7.96
Birth weight (kg)	2.41 ± 0.41	1.59 - 3.23
Measured light irradiance (μW/cm²/nm)	71.8 ± 11.45	48.9 - 83.5

Table 2. Hemoglobin at the initiation of phototherapy and change in total serum bilirubin concentration during phototherapy.

	Mean ± SD	**Range**
Hemoglobin (g/dl)	16.59 ± 2.75	11.09 - 22.09
Hematocrit (%)	45.78 ± 6.18	33.42 - 58.14
TsB_0 (mg/dl)	16.2 ± 6.92	2.36 - 30.04
TsB_{24} (mg/dl)	13.15 ± 3.76	5.63 - 20.67
$\Delta TsB_{0\text{-}24}$ (mg/dl)	5.21 ± 3.05	-

TsB_0, Total serum bilirubin concentration at the start of phototherapy; TsB_{24}, Total serum bilirubin concentration after 24 hours of phototherapy; $\Delta TsB_{0\text{-}24}$, change in total serum bilirubin concentration during initial 24 hours (difference between TsB_0 and TsB_{24}).

Table 3. Linear regression analysis and pearson correlation.

	R	R^2	Coefficient (95% CI)	p-value
Hemoglobin (gm/dl)	−0.035	0.001	−0.286 - 0.153	0.005
Hematocrit (%)	0.055	0.003	−0.050 - 0.143	0.023
TsB_0 (mg/dl)	0.054	0.003	−0.046 - 0.127	0.035
Postnatal age	−0.030	0.001	−0.458 - 0.268	0.607
Birth weight	−0.048	0.002	−2.043 - 0.838	0.411

R, Pearson correlation; R^2, regression; CI, confidence interval; TsB_0, Total serum bilirubin concentration at the start of phototherapy.

4. Discussion

The results of current study showed a weak negative association of hemoglobin with $TsB_{0\text{-}24}$ with significant p-value. To the best of our knowledge only one clinical study done *in vivo* reported hemoglobin as a competitor of bilirubin during phototherapy [1]. On the other hand Granati *et al.* did *in vitro* study and reported significant increase in degradation of bilirubin as the hematocrit level declined during phototherapy but he did not find any of such association *in vivo* [11]. The fact that hemoglobin compete with bilirubin to absorb phototherapy light was confirmed *in vitro* by Lamola *et al*., he used a semi-empirical skin model which showed the competition of hemoglobin with bilirubin and this increased if wavelength increased 400 nm to 460 nm [7]. This finding is also supported *in vitro* by Linfield *et al.* [8].

Looking over the theoretical perspective, the skin capillaries are the primary site for bilirubin isomerization [12] [13], favored by the current study as well. It is observed clinically that skin discoloration of jaundiced neonates reduced during initial hours of phototherapy, this gives a clue that the isomerization of Z-Z-bilirubin to photobilirubin mainly occurs extravascularly in skin. Due to this finding the neonates were changed from prone to supine position during phototherapy [14]. Few of the studies are contradictory to this point and reported that the efficacy of phototherapy is independent of neonate's position because of underlying intravascular mechanism and this can be confirm by measuring photobilirubin in plasma, 15 minutes after the initiation of phototherapy [12] [15] [16] [17]. This finding is supported by the current study as inverse association of hemoglobin with efficacy of phototherapy could be due to intravascular isomerization of bilirubin.

The hemoglobin influences the efficacy of phototherapy by absorbing the light so its effect on degrading hyperbilirubinemia is not accomplished [18]. Mreihil *et al.* gave intensive phototherapy to 36 patients and measured hemoglobin, 4Z, and 15E photoisomers. By doing post-hoc analysis he confirmed a negative association of hemoglobin with Z-E-bilirubin percentage after 15, 30 and 60 minutes but this association disappeared later on. He further explained that after 60 minutes, an equilibrium generated between Z-Z-bilirubin and Z-E-bilirubin in plasma which was not affected by hemoglobin concentration [13]. However,

current study reported inverse correlation between hemoglobin and bilirubin after 24 hours of phototherapy this could be due to the fact that E-Z-lumirubin is the important isomer for lowering bilirubin while there is no existence of equlibirum theory between Z-Z-bilirubin and Z-E-bilirubin, as favored by few other studies [6] [18] [19].

From a clinical point of view, the current study is important for the physician in treating neonates with hyperbilirubinemia by using phototherapy. Neonates having high hemoglobin concentration need phototherapy for longer duration, with higher light irradiance and larger exposure of body surface-area as compared to neonates with lower concentration of hemoglobin. The main limitation of the current study is that high hemoglobin produces more bilirubin which could show a negative correlation between hemoglobin and $\Delta TsB_{0\text{-}24}$. The strengths of the current studies are that the study participants were homogenous and healthy except the hyperbilirubinemia and all the instruments including hematology analyzer and clinical chemistry analyzer were calibrated before performing test.

5. Conclusion

Current study found a significant effect of hemoglobin on efficacy of phototherapy, as the change in total serum bilirubin concentration was inversely correlated with the level hemoglobin. This is important for the physician in treating neonates with hyperbilirubinemia by using phototherapy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Donneborg, M.L., Vandborg, P.K., Hansen, B.M., Rodrigo-Domingo, M. and Ebbesen, F. (2017) The Impact of Hemoglobin on the Efficacy of Phototherapy in Hyperbilirubinemic Infants. *Pediatric Research*, **82**, 947. https://doi.org/10.1038/pr.2017.186

[2] Maisels, M. (2015) Sister Jean Ward, Phototherapy, and Jaundice: A Unique Human and Photochemical Interaction. *Journal of Perinatology*, **35**, 671. https://doi.org/10.1038/jp.2015.56

[3] Ebbesen, F., Hansen, T.W. and Maisels, M.J. (2017) Update on Phototherapy in Jaundiced Neonates. *Current Pediatric Reviews*, **13**, 176-180. https://doi.org/10.2174/1573396313666170718150056

[4] Ebbesen, F., Madsen, P.H., Vandborg, P.K., Jakobsen, L.H., Trydal, T. and Vreman, H.J. (2016) Bilirubin Isomer Distribution in Jaundiced Neonates during Phototherapy with LED Light Centered at 497 nm (Turquoise) vs. 459 nm (Blue). *Pediatric Research*, **80**, 511. https://doi.org/10.1038/pr.2016.115

[5] Mostovnikov, V.A., Mostovnikova, G.R. and Plavski, V.Y. (1995) Spectral and Photochemical Parameters Which Define the Higher Efficacy of Laser Phototherapy in Hyperbilirubinemia in Newborns. 5*th International Conference on Laser Applica-*

tions in Life Sciences, Vol. 2370, 558-561. https://doi.org/10.1117/12.197489

[6] Onishi, S., Isobe, K., Itoh, S., Manabe, M., Sasaki, K., Fukuzaki, R., *et al.* (1986) Metabolism of Bilirubin and Its Photoisomers in Newborn Infants during Phototherapy. *The Journal of Biochemistry*, **100**, 789-795. https://doi.org/10.1093/oxfordjournals.jbchem.a121772

[7] Lamola, A.A., Bhutani, V.K., Wong, R.J., Stevenson, D.K. and McDonagh, A.F. (2013) The Effect of Hematocrit on the Efficacy of Phototherapy for Neonatal Jaundice. *Pediatric Research*, **74**, 54. https://doi.org/10.1038/pr.2013.67

[8] Linfield, D.T., Lamola, A.A., Mei, E., Hwang, A.Y., Vreman, H.J., Wong, R.J., *et al.* (2016) The Effect of Hematocrit on *in Vitro* Bilirubin Photoalteration. *Pediatric Research*, **79**, 387. https://doi.org/10.1038/pr.2015.240

[9] Curran, S., McMurdy, J.W., Carr, S.R., Muratore, C.S., O'Brien, B.M., Crawford, G.P., *et al.* (2010) Reflectance Spectrometry for Real-Time Hemoglobin Determination of Placental Vessels during Endoscopic Laser Surgery for Twin-to-Twin Transfusion Syndrome. *Journal of Pediatric Surgery*, **45**, 59-64. https://doi.org/10.1016/j.jpedsurg.2009.10.009

[10] Sikurova, L., Balis, P. and Zvarik, M. (2011) Penetration of Laser Light through Red Blood Cell Ghosts. *Journal of Photochemistry and Photobiology B*: *Biology*, **103**, 230-233. https://doi.org/10.1016/j.jphotobiol.2011.03.015

[11] Granati, B., Felice, M., Fortunato, A., Giancola, G. and Rubaltelli, F.F. (1983) Sites of Action of Light during Phototherapy. *Neonatology*, **43**, 1-8. https://doi.org/10.1159/000241630

[12] Donneborg, M., Knudsen, K.B. and Ebbesen, F. (2010) Effect of Infants' Position on Serum Bilirubin Level during Conventional Phototherapy. *Acta Paediatrica*, **99**, 1131-1134. https://doi.org/10.1111/j.1651-2227.2010.01885.x

[13] Mreihil, K., Madsen, P., Nakstad, B., Benth, J.Š., Ebbesen, F. and Hansen, T.W.R. (2015) Early Formation of Bilirubin Isomers during Phototherapy for Neonatal Jaundice: Effects of Single vs. Double Fluorescent Lamps vs. Photodiodes. *Pediatric Research*, **78**, 56. https://doi.org/10.1038/pr.2015.61

[14] Hansen, T.W.R. (1996) Therapeutic Approaches to Neonatal Jaundice: An International Survey. *Clinical Pediatrics*, **35**, 309-316. https://doi.org/10.1177/000992289603500604

[15] Bhethanabhotla, S., Thukral, A., Sankar, M., Agarwal, R., Paul, V. and Deorari, A. (2013) Effect of Position of Infant during Phototherapy in Management of Hyperbilirubinemia in Late Preterm and Term Neonates: A Randomized Controlled Trial. *Journal of Perinatology*, **33**, 795. https://doi.org/10.1038/jp.2013.54

[16] Chen, C.M., Liu, S.H., Lai, C.-C., Hwang, C.C. and Hsu, H.-H. (2002) Changing Position Does Not Improve the Efficacy of Conventional Phototherapy. *Acta Paediatrica Taiwanica*, **43**, 255-258.

[17] Mohammadzadeh, A., Bostani, Z., Jafarnejad, F. and Mazloom, R. (2004) Supine versus Turning Position on Bilirubin Level during Phototherapy in Healthy Term Jaundiced Neonates. *Saudi Medical Journal*, **25**, 2051-2052.

[18] Lamola, A.A. (2016) A Pharmacologic View of Phototherapy. *Clinics in Perinatology*, **43**, 259-276. https://doi.org/10.1016/j.clp.2016.01.004

[19] Okada, H., Masuya, K., Yasuda, S., Okubo, K., Kawada, K., Kusaka, T., *et al.* (2005) Developmental Changes in Serum Half-Life of (EZ)-Cyclobilirubin. *Early Human Development*, **81**, 619-622. https://doi.org/10.1016/j.earlhumdev.2005.03.014

International Journal of Clinical Medicine, 2019, 10, 531-542
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Percentage Change on FDG-PET/CT Predicts Complete Response to Neoadjuvant Radiochemotherapy in Esophageal Cancer

Esther Jimenez-Jimenez[1*], Irene Ortiz[2], Neus Aymar[2], Raquel Roncero[2], Pedro Mateos[3], Marta Gimenez[4], Jose Pardo[2], Sebastià Sabater[1]

[1]Radiation Oncology Department, Complejo Hospitalario Universitario, de Albacete, Spain
[2]Radiation Oncology Department, Hospital Universitari Son Espases, Palma de Mallorca, Spain
[3]Medical Physics Department, Hospital Universitari Son Espases, Palma de Mallorca, Spain
[4]Nuclear Medicine Department, Hospital Universitari Son Espases, Palma de Mallorca, Spain
Email: *estherjj@hotmail.com

How to cite this paper: Jimenez-Jimenez, E., Ortiz, I., Aymar, N., Roncero, R., Mateos, P., Gimenez, M., Pardo, J. and Sabater, S. (2019) Percentage Change on FDG-PET/CT Predicts Complete Response to Neoadjuvant Radiochemotherapy in Esophageal Cancer. *International Journal of Clinical Medicine*, **10**, 531-542.
https://doi.org/10.4236/ijcm.2019.1010043

Received: September 23, 2019
Accepted: October 19, 2019
Published: October 22, 2019

Abstract

Objective: We evaluated whether the changes in FDG-PET/CT uptake between pre/and post-treatment might predict a complete response in esophageal cancer (EC). Patients and Methods: Fifty-six patients with 2 PET-CTs studies were evaluated retrospectively. Images were evaluated qualitatively and semiquantitatively (SUVs). Patients were classified as persistence of disease, complete metabolic response and unspecific FDG uptake. The SUVmax values and percentages of change in SUV were measured. Results: A significant difference was found between the persistence group and the non-persistence group regarding the median percentage change in SUVtumor (72.95% vs. 54.12%; p = 0.04) and regarding the percentage change in SUVnode (89.91% vs. 59.91%, p = 0.04). In patients treated with radiochemotherapy (RCTX), a significant difference was found between the persistence group and the no persistence group regarding the percentage change in SUVtumor (58.02% vs. 78.59%). Overall survival rate was related to the percentage of change in the SUVtumor. The group of ≥75% of change SUVtumor showed a median survival of 37.32 months (IC: 95% = 49.93 - 24.70) and the group of <75% of change SUVtumor showed a median survival of 18.39 months (IC: 95% = 25.14 - 11.65) (p = 0.04). In patients with metastatic relapse, a significant difference was found regarding the percentage change in SUVnode (94.63% vs. 74.09%). Conclusion: Our study provides evidence that the percentage change in the SUVmax is a predictor of the response to neoadjuvant treatment in patients with EC. When SUVmax decreases by 72.95% or more, the patient is

likely a complete responder.

Keywords

PET/CT, Esophageal, Response, SUV, Percentage

1. Introduction

Esophageal cancer (EC) is the sixth leading cause of cancer-related mortality worldwide [1] and most patients have locally advanced disease at diagnosis. Currently, the standard of care for patients with resectable locally advanced esophageal or esophagogastric junctional cancer is neoadjuvant radiochemotherapy (RCTX) or chemotherapy (CTX) followed by surgical resection [2] [3]. However, for squamous cell carcinoma, two randomized trials have reported no survival benefits of surgery [4] [5] and for this reason, there is a growing interest in selecting patients who could be treated by non-invasive tests rather than surgery.

Nowadays, the value of FDG-PET/CT for restaging after induction treatment is not clear and international guidelines differ in their recommendations. Limited studies suggest that PET/CT imaging detects distant metastases following induction RCTX [6] and some centers routinely order a post-induction PET/CT approximately four weeks after the completion of treatment as a method to assess for distant metastatic disease. In addition, post-induction-therapy FDG-PET provides information on the metabolic response in the primary tumor that may be clinically useful in the selection of subsequent therapy [7]. However, others have failed to find a correlation between the post-RCTX SUV on PET and pathologic response at the time of resection [8].

According to current evidence, the main use of PET-CT in the revaluation of EC may be to recognize which patients are not responding to induction CTX [9]. However, there is limited evidence according to the role of PET-CT after RCTX. For this reason, the aim of this study was to assess the role of FDG-PET/CT in response evaluation to neoadjuvant RCT in the EC and determine whether the changes in FDG-PET/CT uptake between pre/and post-induction treatment could be related to the persistence or not of tumor.

2. Patients and Methods

2.1. Patients

Fifty-six patients with locally advanced EC treated between April 2013 and November 2016 were evaluated retrospectively following approval by the local ethics committee. All patients were required to have staging with a physical examination, endoscopic/ultrasonography and cervical/thoracic/abdominal CT. The eighth edition of the TNM staging was used. All of them had at least 2 FDG-PET/CT studies, one before RCTX with PET/CT1 as a baseline and the

other after treatment (PET/CT2). No patient had metastatic disease at the time of diagnosis. The images were confirmed by histopathology.

2.2. FDG-PET/CT STAGING (PET/CT1)

Image acquisition procedures have been described elsewhere [10]. In summary, all patients fasted for at least 6 h before the FDG-PET/CT examination. They were injected with 4 MBq/kg body weight of 18FDG and then rested for about 1 h in a quiet room before imaging. CT acquisition parameters were: 100 kV, 80 - 250 mA, slice thickness of 2.5 mm. It was used both for the attenuation correction of PET data and to locate the FDG uptake in PET images. PET scanning was performed covering the same axial range for 2 min per bed position (total of 3 - 5 bed positions). Both PET and CT acquisition were performed during free breathing. Data were reconstructed using an ordered subset expectation maximization (OSEM), 3D algorithm (3D Iterative) and attenuation correction derived from CT data. The average acquisition time of the images was between 12 - 16 minutes. The maximal standardized tumor uptake values (SUVmax) were normalized by lean body mass (LBM). The FDG-PET image was normalized to the physiological FDG uptake in the liver and, after identifying the primary tumor as a region of interest (ROI), the SUVmax was obtained from the most metabolically active lesion.

2.3. Treatment and Follow-Up

Patients underwent RCTX or neoadjuvant CTX. The radiotherapy (RTX) was delivered using a three-dimensional conformal technique. Median radiation dose was 50.4 Gy. The gross tumor volume (GTV) was delineated according to Visual analysis of nuclear medicine physicians [11] and the clinical target volume (CTV) according to the ESTRO and EORTC-RTOG recommendations [12]. It consisted of the primary lesions and regional nodal regions with a 3 to 5 cm craniocaudal and 1 to 2 cm circumferential margin. The CTX treatment scheme was CDDP-5FU or EOX according to histology. Surgical resection was performed on 16 patients. The decision on local resectability was taken by a multidisciplinary committee and depended on tumor infiltration into neighboring structures, distant metastases and absence/presence of uptake of FDG-PET/CT. The histopathological response was measured according to the Mandard tumor regression grade scoring system [13]. Patients were followed up every 3 - 6 months. Locoregional control was defined as "time until proven recurrence". Overall survival was defined as "time until death".

2.4. FDG-PET/CT Re-Evaluation (PET/CT2)

All patients were re-evaluated by PET/CT2, in the same nuclear medicine department and PET/CT2 was carried out 3 months after RCTX. The response by PET/CT was measured in absolute terms and as a percentage reduction in SUVmax. No other assessment parameters were used (such as metabolic tumor

volume, total lesion glycolysis or SUVmean) because they are not usually used in clinical practice.

2.5. Image Interpretation and Analysis

Image analysis procedures have already been described [14]. The PET images were evaluated qualitatively (visual inspection) and semiquantitatively (SUVs). PET/CT2 scans were interpreted and correlated with clinical information and the initial PET/CT1. Patients were classified into 3 groups: persistence of disease (PET/CT2 shows loco/regional disease or progression), complete metabolic response (SUVmax on PET/CT2 ≤ 3) and unspecific FDG uptake. A team of experienced nuclear medicine physicians and radiologists defined whether persistence or non-persistence of disease. They evaluated tumor recurrence at the anastomosis site of the resected primary tumor based on a visual analysis on the PET-CT scan. The following characteristics were used to describe FDG uptake on fused PET-CT images: the asymmetric of the uptake and the extend of FDG uptake, whereas malignant uptake tends to be focal, eccentric, and benign uptake (due to RT or reflux esophagitis) tends to be diffuse and concentric. Suspect lesions were confirmed by biopsy or by radiological and clinical follow-up. Patients who underwent surgery were not classified in these groups.

The SUVmax values of the esophageal tumor and lymph nodes on PET/CT images were collected. Percentages of change in SUV were calculated as (PET/CT1 SUV – PET/CT2 SUV) × 100/PET/CT1 SUV. There are studies that have divided these into subcategories according to percentage change but there are no generally accepted threshold values [15] [16]. In the present study, all uptakes were divided into two groups according to the percentage of change in the SUV (≥75% and <75%). We used this value because we found the persistence group had a percentage of change in SUV tumor of 72.95%.

2.6. Statistical Analysis

Overall survival (OS) and progression-free survival (PFS) were calculated by Kaplan-Meier survival analysis, and comparison between groups was made by the log-rank test. Univariate and multivariate Cox proportional models were used and a value of $p < 0.05$ was considered to be statistically significant. SPSS v. 22.00 was used for statistical analysis.

3. Results

3.1. Patient Characteristics

Median follow-up of patients was 20.4 months (range: 4.2 - 56.5 weeks). **Figure 1** shows a profile of this studyin which 56 patients were assessed (men = 47, women = 9, with median age 62.7 years (± 8.2). The patients' characteristics are provided in **Table 1**. Sixteen patients (28.6%) underwent surgery according to the response and 40 patients (71.4%) were treated without surgery.

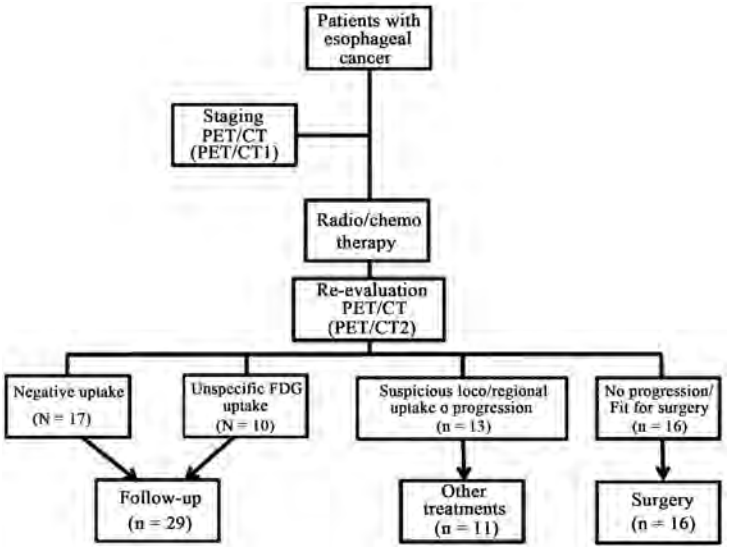


Figure 1. Study population and design.

Table 1. Patients' characteristics and findings on PET/CT1.

Characterisits	Value
Numer of patients	**56**
Age (years) (median ± DS)	62.7 ± 8.2
Gender	
Male	47 (83.9%)
Female	9 (16.1%)
Location	
Cervical	11 (19.6%)
Thoracic	20 (35.7)
Lower	25 (44.6%)
Histology	
Squamous	42 (75%)
Adenocarcinoma	13 (23.2%)
Grade	
G1-2	7 (12.5%)
G3-4	17 (30.4%)
Unknown	32 (57.1%)
Treatment	
Radio-chemo	47 (83.9%)
Chemo	9 (16.1%)
Tumor +/− (PET/CT1)	
PET-T+	55(98.2%)
PET-T−	1 (1.8%)
Node +/− (PET/CT1)	
PET-N+	21 (37.5%)
PET-N−	35 (62.5%)
Number involved nodes	
N0	19 (33.9%)
N1 (1 - 2 nodes)	27 (48.2%)
N2 (3 - 6 nodes)	10 (17.9%)
N3 (>7 nodes)	0

3.2. Findings on PET/CT1

Fourteen patients were found to have stage II disease and 42 patients had stage III. The mean SUVmax values of the esophageal tumor were 12.08 (range 0 - 30.2) and 37 patients had clinically involved lymph nodes on PET/CT images: N0 (33.9%), N1 (48.2%), N3 (17.9%). The mean SUVmax values of the metastatic lymph nodes were 3.94 (range 0 - 16.1). Details of findings on PET/CT1 are shown in Table 1.

3.3. Findings on PET/CT2

- **Post-treatment tumoral and node evaluation**

Thirteen patients (23.2%) were found to have persistence of disease, a complete metabolic response was shown in 17 patients (30.4%) and 10 patients (17.9%) were found to have unspecific FDG uptake (with/without residual disease). 16 patients (28.6%) underwent an operation.

The mean SUVmax values of the esophageal tumor were 3.8 (range 0 - 24.7). The mean SUVmax values of the metastatic lymph nodes were 0.8 (range 0 - 8.8). 13 patients had clinically involved lymph nodes on PET/CT2 images: N0 (76.8%), N1 (19.6%), none with N3.

All patients had a mean decrease in tumor uptake of 68.4% (SD: 33.2%) and 55 patients had a mean decrease in node uptake of 81.6% (SD: 33.6%), except 1 patient who had an increased node uptake. There was no correlation between the decrease in tumor or node uptake and the time between PET1 and PET2.

- **Changes between PET/CT1 and PET/CT2**

Patients were categorized according to follow-up into 2 groups: patients with persistence of disease or progression (Persistence) and patients with disease-free time (No persistence). Suspect lesions were confirmed. Patients who underwent surgery were excluded. These results were compared with the percentage of tumor change between both tests. A significant difference was found between the persistence group and the non-persistence group regarding the percentage change in SUVtumor (72.95% vs 54.12%) and post-treatment SUVtumor, and regarding the percentage change in SUVnode (89.91% vs 59.91%) and post-treatment SUVnode (Table 2).

Secondly, we divided the patients into 2 groups according to treatment received: RCTX or only CTX. No patient was treated with the only RTX. In patients treated with RCTX, a significant difference was found between the persistence group and the no persistence group regarding the percentage change in

Table 2. Comparison between persistence and non-persistence on PET/CT imaging.

	Pre-SUV tumor	Post-SUV tumor	% change SUV tumor	Pre-SUV node	Post-SUV node	% change SUV node
Persistence (+) (n = 13)	12.5 ± 6.15	6.02 ± 6.11	54.12% ± 26.68%	5.05 ± 3.69	2.31 ± 3.24	59.91% ± 47.25%
Persistence (−) (n = 43)	11.95 ± 5.96	3.13 ± 3.43	72.95% ± 30.15%	3.60 ± 3.99	0.43 ± 1.28	89.51% ± 23.91%
	p = 0.780	p = 0.03	p = 0.04	p = 0.271	p = 0.08	p = 0.04

SUVtumor (58.02% vs 78.59%). Regarding percentage change in SUVnode no difference was found (Table 3). Regarding the percentage change in SUVnode, no differences were found.

3.4. Pathological Response

Sixteen patients underwent surgical resection. Pathological findings were: G1 (absence of residual tumor): 3.6%, G2 (rare residual tumor over a lot of fibrosis): 5.4%, G3 (predominate fibrosis over residual tumor): 1.8%, G4 (more residual tumor than fibrosis) 12.5%, G5 (absence of response): 3.6% and 1 unknown. Six patients had been treated with CTX and 10 patients with RCTX. Nine patients were adenocarcinoma and 7 patients squamous carcinoma. Most of them were gastroesophageal junction tumors (13/16). According to the degree of tumor response, no differences were observed between PET/CT1 and PET/CT2. However, we observed differences in SUVnode of PET/CT1 between tumors with a low degree or high degree of response to treatment ($p = 0.016$).

3.5. Overall Survival and Outcomes

Mean overall survival time was 24.18 months; 11.82 months in patients with persistence on PET/CT2 and 29.27 months in patients without persistence on PET/CT2. Patients who underwent surgery were excluded.

On univariate analysis, the overall survival rate was related to the percentage of change in the SUVtumor and SUVnode. The group of ≥75% of change SUVtumor showed a median survival of 37.32 months (IC: 95% = 49.93 - 24.70) and the group of <75% of change SUVtumor showed a median survival of 18.39 months (IC: 95% = 25.14 - 11.65) ($p = 0.04$; Figure 2). However, the group of ≥75% change SUVnode showed a median survival of 20.56 months (IC: 95% = 25.56 - 14.57) and the group of <75% change SUVnode showed a median survival of 17.24 months (IC: 95% = 27.60 - 60.87) ($p = 0.14$: Figure 2).

Finally, we compared differences in SUVmax values of the esophageal tumor and lymph nodes between PET/CT1 and PET/CT2 according to the pattern of relapse. In patients with metastatic relapse, a significant difference was found regarding the percentage change in SUVnode (94.63% vs. 74.09%).

4. Discussion

Currently, the value of FDG-PET/CT in treatment response assessment of EC is not clear, above all after treatment with RCTX. Some studies indicate PET-CT is

Table 3. Comparison between treatments received on PET/CT imaging.

		Pre-SUV tumor	Post-SUV tumor	% change SUV tumor	Pre-SUV node	Post-SUV node	% change SUV node
Radio/chemotherapy (n = 47)	Persistence (+)	11.07 ± 3.39	4.37 ± 2.63	58.02 % ± 26.53%	5.09 ± 3.39	2.55 ± 3.31	59.91% ± 47.05%
	Persistence (−)	11.54 ± 5.44	2.44 ± 2.76	78.59 % ± 26.25%	3.69 ± 4.11	0.42 ± 1.31	89.5% ± 25.12%
		p = 0.78	**p = 0.04**	**p = 0.02**	p = 0.33	p = 0.07	p = 0.12

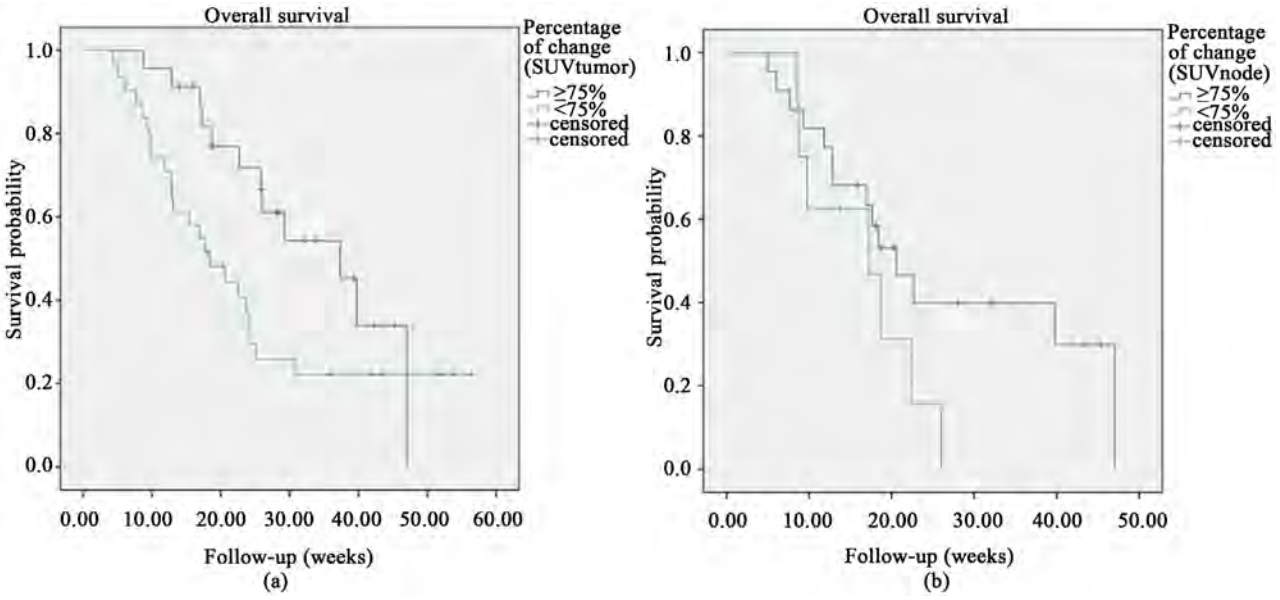


Figure 2. Overall survival rate for percentage of change SUVtumor (a); p = 0.004, and for percentage of change SUVnode (b), p = 0.14.

useful in the reevaluation of treatment [17] and could even be useful in diagnosing a metabolic complete response. The MUNICON trial confirmed the usefulness of an early metabolic evaluation in patients with ADC treated only with neoadjuvant CT [9]. However, other studies have not been able to find a correlation between post-treatment SUV and the pathological response at the time of resection [8]. Even the guidelines differ in their recommendations, NCCN® guidelines recommend routinely performing a reassessment PET-CT after 4 weeks after completing the treatment, however UPTODATE® recommend not using PET-CT after treatment to select surgical patients until there is no more evidence in this regard.

In addition, although it is currently considered that the optimal treatment of EC is neoadjuvant RCTX or CT followed by surgery, there are studies have questioned the need for esophagectomy after achieving a complete pathological response after neoadjuvant treatment, especially in SCC [7], so non-invasive tests are needed to select patients who respond to initial treatment. A systematic review of 2017 with 697 patients concluded that the available data are contradictory to conclude that FDG-PET has a predictive value in the EC, although there were relevant results that would support its use for the individual therapeutic decision [18].

For this reason, our aim was to analyze the role of FDG-PET/CT in the evaluation of the response to neoadjuvant RCT in the EC. Our results indicate that changes between PET1 and PET2 to predict complete metabolic response. A significant difference was found between the persistence group and the non-persistence group regarding the percentage change in SUVtumor (72.95% vs. 54.12%) and post-treatment SUVtumor, and regarding the percentage change in SUVnode (89.91% vs. 59.91%) and post-treatment SUVnode. This difference

was maintained between patients receiving RCT, despite the increased uptake that can be caused by inflammation by RTX [19]. In addition, group of patients with ≥75% of change SUVtumor showed a better overall survival than group of patients with <75%. The selection of the cut-off value of the percentage of tumor change may be important. The range of cut-off values varies widely by different studies, but none have been considered as optimal [20]. Smithers *et al.* [15] observed the mean percentage change in SUV in the histological responders group was −56.8% (SD 29) and in the non-responders −27.8% (SD 32.1) (P = 0.035). Brücher *et al.* [16] concluded that in responders FDG uptake decreased by 72% ± 11% and in non-responders it decreased by only 42% ± 22%. We use this value in our study because we found the persistence group had a percentage of change in SUVtumor of 72.95%.

There are studies that indicate that a complete pathological response is associated with better survival [21] however, studies only provide conflicting results regarding the utility of FDG-PET images to predict the histopathological response of the primary tumor after neoadjuvant treatment. In addition, optimal PET parameters have not been established to assess the response to treatment, which provides an additional confounding factor. Arnett *et al.* [8] found no correlation between tumor pathological response and PET findings although, similar to our results, patients with a large tumor reduction after RCT had better results. Otherwise, Cerfolio *et al.* [22] supported the usefulness of SUVmax as a predictor of pathological response in a study with 86 patients. Similar to our results, they concluded that patients with reduction of 64% in FDG-PET uptake could predict a complete pathological response, but no relationship with survival was found.

Neoadjuvant treatment could also be a factor to consider. It is known that CTX and RTX affect carbohydrate metabolism in tumor cells. In vitro studies have demonstrated a transient increase in early FDG uptake after exposure to CTX [23], however a similar increase has not been described in vivo. Furthermore, the RTX directly affects the metabolic activity of tumor cells and induces an inflammatory reaction with increased FDG uptake [24], which can mask a metabolic response. Therefore, radiation-induced esophagitis could affect the measurement of FDG uptake by the tumor. However, in our study the difference between the percentage of change between patients with or without persistence of disease was maintained in the group treated with RCTX. In order to minimize these unwanted effects, PET/CT should be carried out 8 - 12 weeks after RCTX.

It is important to note that our study has several limitations, including its retrospective nature and its analysis of a small number of patients with heterogeneous characteristics. Also, most of our patients were SCC and were treated with RCTX without surgery, because they had a histopathologically confirmed complete response. This was caused because the patients were systematically collected from the usual clinical practice, without attending to the histology or neoadjuvant treatment received. Therefore, results cannot be compared with other studies in which patients were subsequently operated. In our hospital, pa-

tients were selected for the high percentage of PET-CT response and for the clinical information of other tests such as endoscopy or biopsy of the suspicious area.

Another limitation might be the method of metabolic uptake measurements. According to Vallbohmer *et al.* [25] many factors influence SUV measurements, such as size, measurement duration, plasma glucose concentration, recovery coefficient, partial volume and ROI selection. Other parameters than SUVmax could have been analyzed as MTV (metabolic tumorvolumen), TLG (total lesion glycolysis) or SUVmean, but our patients and their PET-CT were collected from the usual clinical practice, and SUVmax is the value usually used in our hospital.

5. Conclusion

In conclusion, our findings indicate that PET/CT imaging might be a standard component of the reevaluation of all locally advanced EC patients, especially to identify patients with or without persistence of tumor disease. The present study provides more evidence of the ability of changes between PET/CT1 vs. PET/CT2 to evaluate tumor response after RCTX. In the future, changes produced between both PET/CTs could be used to decide which patients should undergo surgery. More studies are needed to evaluate post-treatment changes in PET/CT in EC.

Support/Funding

No Financial Support/Funding Statement.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Pennathur, A., Gibson, M.K., Jobe, B.A. and Luketich, J.D. (2013) Oesophageal Carcinoma. *The Lancet*, **381**, 400-412. https://doi.org/10.1016/S0140-6736(12)60643-6

[2] Sjoquist, K.M., Burmeister, B.H., Smithers, B.M., *et al.* (2011) Survival after Neoadjuvant Chemotherapy or Chemoradiotherapy for Resectable Oesophageal Carcinoma: An Updated Meta-Analysis. *The Lancet Oncology*, **12**, 681-692. https://doi.org/10.1016/S1470-2045(11)70142-5

[3] Shapiro, J., van Lanschot, J.J.B., Hulshof, M., *et al.* (2015) Neoadjuvant Chemoradiotherapy plus Surgery versus Surgery Alone for Oesophageal or Junctional Cancer (CROSS): Long-Term Results of a Randomised Controlled Trial. *The Lancet Oncology*, **16**, 1090-1098. https://doi.org/10.1016/S1470-2045(15)00040-6

[4] Stahl, M., Stuschke, M., Lehmann, N., *et al.* (2005) Chemoradiation with and without Surgery in Patients with Locally Advanced Squamous Cell Carcinoma of the Esophagus. *Journal of Clinical Oncology*, **23**, 2310-2317. https://doi.org/10.1200/JCO.2005.00.034

[5] Bedenne, L., Michel, P., Bouche, O., *et al.* (2007) Chemoradiation Followed by Sur-

gery Compared with Chemoradiation Alone in Squamous Cancer of the Esophagus: FFCD 9102. *Journal of Clinical Oncology*, **25**, 1160-1168. https://doi.org/10.1200/JCO.2005.04.7118

[6] Bruzzi, J.F., Swisher, S.G., Truong, M.T., *et al.* (2007) Detection of Interval Distant Metastases: Clinical Utility of Integrated CT-PET Imaging in Patients with Esophageal Carcinoma after Neoadjuvant Therapy. *Cancer*, **109**, 125-134. https://doi.org/10.1002/cncr.22397

[7] Monjazeb, A.M., Riedlinger, G., Aklilu, M., *et al.* (2010) Outcomes of Patients with Esophageal Cancer Staged with [(1)(8)F]fluorodeoxyglucose Positron Emission Tomography (FDG-PET): Can Postchemoradiotherapy FDG-PET Predict the Utility of Resection? *Journal of Clinical Oncology*, **28**, 4714-4721. https://doi.org/10.1200/JCO.2010.30.7702

[8] Arnett, M.K., Macintosh, E.M., James, S.E., Nathan, M.A., Shen, K.R., Ravi, K., Neben Wittich, M.A., Haddock, M.G. and Hallemeier, C.L. (2017) Utility of (18)F-FDG PET for Predicting Histopathologic Response in Esophageal Carcinoma Following Chemoradiation. *Journal of Thoracic Oncology*, **12**, 121-128. https://doi.org/10.1016/j.jtho.2016.08.136

[9] Lordick, F., Ott, K., Krause, B.J., *et al.* (2007) PET to Assess Early Metabolic Response and to Guide Treatment of Adenocarcinoma of the Oesophagogastric Junction: The MUNICON Phase II Trial. *The Lancet Oncology*, **8**, 797-805. https://doi.org/10.1016/S1470-2045(07)70244-9

[10] Jimenez-Jimenez, E., Mateos, P., Aymar, N., *et al.* (2018) Radiotherapy Volume Delineation Using 18F-FDG-PET/CT Modifies Gross Node Volume in Patients with Oesophageal Cancer. *Clinical and Translational Oncology*, **20**, 1460-1466. https://doi.org/10.1007/s12094-018-1879-3

[11] Leong, T., Everitt, C., Yuen, K., *et al.* (2006) A Prospective Study to Evaluate the Impact of FDG-PET on CT-Based Radiotherapy Treatment Planning for Oesophageal Cancer. *Radiotherapy & Oncology*, **78**, 254-261. https://doi.org/10.1016/j.radonc.2006.02.014

[12] Matzinger, O., Gerber, E., Bernstein, Z., *et al.* (2009) EORTC-ROG Expert Opinion: Radiotherapy Volume and Treatment Guidelines for Neoadjuvant Radiation of Adenocarcinomas of the Gastroesophageal Junction and the Stomach. *Radiotherapy & Oncology*, **92**, 164-175. https://doi.org/10.1016/j.radonc.2009.03.018

[13] Mandard, A.M., Dalibard, F., Mandard, J.C., *et al.* (1994) Pathologic Assessment of Tumor Regression after Preoperative Chemoradiotherapy of Esophageal Carcinoma. Clinicopathologic Correlations. *Cancer*, **73**, 2680-2686. https://doi.org/10.1002/1097-0142(19940601)73:11<2680::AID-CNCR2820731105>3.0.CO;2-C

[14] Jimenez-Jimenez, E., Mateos, P., Ortiz, I., *et al.* (2019) Nodal FDG-PET/CT Uptake Influences Outcome and Relapse Location among Esophageal Cancer Patients Submitted to Chemotherapy or Radiochemotherapy. *Clinical and Translational Oncology*, **21**, 1159-1167. https://doi.org/10.1007/s12094-019-02038-6

[15] Smithers, B.M., Couper, G.C., Thomas, J.M., *et al.* (2008) Positron Emission Tomography and Pathological Evidence of Response to Neoadjuvant Therapy in Adenocarcinoma of the Esophagus. *Diseases of the Esophagus*, **21**, 151-158. https://doi.org/10.1111/j.1442-2050.2007.00732.x

[16] Brucher, B.L., Weber, W., Bauer, M., *et al.* (2001) Neoadjuvant Therapy of Esophageal Squamous Cell Carcinoma: Response Evaluation by Positron Emission Tomography. *Annals of Surgery*, **233**, 300-309.

https://doi.org/10.1097/00000658-200103000-00002

[17] Brucher, B.L., Swisher, S.G., Konigsrainer, A., *et al.* (2009) Response to Preoperative Therapy in Upper Gastrointestinal Cancers. *Annals of Surgical Oncology*, **16**, 878-886. https://doi.org/10.1245/s10434-009-0315-x

[18] Cremonesi, M., Garibaldi, C., Timmerman, R., *et al.* (2017) Interim (18)F-FDG-PET/CT during Chemo-Radiotherapy in the Management of Oesophageal Cancer Patients. A Systematic Review. *Radiotherapy and Oncology*, **125**, 200-212. https://doi.org/10.1016/j.radonc.2017.09.022

[19] Culverwell, A.D., Scarsbrook, A.F. and Chowdhury, F.U. (2011) False-Positive Uptake on 2-[(1)(8)F]-fluoro-2-deoxy-D-glucose (FDG) Positron-Emission Tomography/Computed Tomography (PET/CT) in Oncological Imaging. *Clinical Radiology*, **66**, 366-382. https://doi.org/10.1016/j.crad.2010.12.004

[20] Malik, V., Lucey, J.A., Duffy, G.J., *et al.* (2010) Early Repeated 18F-FDG PET Scans during Neoadjuvant Chemoradiation Fail to Predict Histopathologic Response or Survival Benefit in Adenocarcinoma of the Esophagus. *Journal of Nuclear Medicine*, **51**, 1863-1869. https://doi.org/10.2967/jnumed.110.079566

[21] Castoro, C., Scarpa, M., Cagol, M., *et al.* (2013) Complete Clinical Response after Neoadjuvant Chemoradiotherapy for Squamous Cell Cancer of the Thoracic Oesophagus: Is Surgery Always Necessary? *Journal of Gastrointestinal Surgery*, **17**, 1375-1381. https://doi.org/10.1007/s11605-013-2269-3

[22] Cerfolio, R.J., Bryant, A.S., Talati, A.A., *et al.* (2009) Change in Maximum Standardized Uptake Value on Repeat Positron Emission Tomography after Chemoradiotherapy in Patients with Esophageal Cancer Identifies Complete Responders. *The Journal of Thoracic and Cardiovascular Surgery*, **137**, 605-609. https://doi.org/10.1016/j.jtcvs.2008.11.016

[23] Haberkorn, U., Morr, I., Oberdorfer, F., *et al.* (1994) Fluorodeoxyglucose Uptake *in Vitro*: Aspects of Method and Effects of Treatment with Gemcitabine. *Journal of Nuclear Medicine*, **35**, 1842-1850.

[24] Schiepers, C., Haustermans, K., Geboes, K., *et al.* (1999) The Effect of Preoperative Radiation Therapy on Glucose Utilization and Cell Kinetics in Patients with Primary Rectal Carcinoma. *Cancer*, **85**, 803-811. https://doi.org/10.1002/(SICI)1097-0142(19990215)85:4<803::AID-CNCR7>3.0.CO;2-T

[25] Vallbohmer, D., Holscher, A.H., Dietlein, M., *et al.* (2009) [18F]-Fluorodeoxyglucose-Positron Emission Tomography for the Assessment of Histopathologic Response and Prognosis after Completion of Neoadjuvant Chemoradiation in Esophageal Cancer. *Annals of Surgery*, **250**, 888-894. https://doi.org/10.1097/SLA.0b013e3181bc9c0d

International Journal of Clinical Medicine, 2019, 10, 543-552
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Arthroscopic Removal of Metallic Suture Anchors Placed after Bankart Repair

Olcay Guler[1*], Mehmet Isyar[2], Selami Cakmak[2], Melih Malkoc[3], Halis Cerci[4], Mahir Mahirogullari[5]

[1]School of Medicine, Department of Orthopaedics and Traumatology, Istanbul Istinye University, Istanbul, Turkey
[2]Department of Orthopaedics and Traumatology, Acıbadem Kadıkoy Hospital, Istanbul, Turkey
[3]School of Medicine, Department of Orthopaedics and Traumatology, Istanbul Medipol University, Istanbul, Turkey
[4]Department of Orthopaedic and Traumatology, Nisa Hospital, Istanbul, Turkey
[5]Memorial Health Group, Department of Orthopaedics and Traumatology, Istanbul, Turkey
Email: *olcayguler77@gmail.com, misyar2003@yahoo.com, selamicakmak@gmail.com, memalkoc@yahoo.com, doblili@hotmail.com, mahirogullari@yahoo.com

How to cite this paper: Guler, O., Isyar, M., Cakmak, S., Malkoc, M., Cerci, H. and Mahirogullari, M. (2019) Arthroscopic Removal of Metallic Suture Anchors Placed after Bankart Repair. *International Journal of Clinical Medicine*, **10**, 543-552.
https://doi.org/10.4236/ijcm.2019.1010044

Received: September 20, 2019
Accepted: October 20, 2019
Published: October 23, 2019

Abstract

Purpose: The aim of this study was to present our surgical outcomes in patients who underwent arthroscopic removal of poorly positioned and/or proud metallic suture anchors applied during or after Bankart repair. **Methods:** A total of 14 patients who underwent open or arthroscopic Bankart repair with an initial presentation of traumatic shoulder instability between January 2010 and January 2017 and admitted to our center with complaints due to poorly positioned and/or proud metallic suture anchors were enrolled. Pre- and intraoperative findings, surgical outcomes and complications were reviewed. Diagnosis of proud or poorly positioned suture anchors was established using magnetic resonance imaging (MRI) of shoulder in five cases, and with shoulder arthroscopy in nine patients. Outcomes were measured by the use of the CONSTANT score and American Shoulder and Elbow Society (ASES) score. **Results:** Eleven male and three female patients with an average age of 29.21 ± 5.78 (range, 20 to 42) were enrolled in the present study. Revision Bankart repair was performed arthroscopically in all patients. The mean follow-up period was 40.4 months, ranging from 18 to 64 months. The preoperative Constant and ASES scores were 68.43 ± 7.05 and 38.3 ± 19.4, respectively. Postoperatively, the scores were 89.64 ± 5.39 and 89.07 ± 3.89, respectively ($p < 0.01$). **Conclusion**: To conclude, arthroscopy may yield an effective surgical option for removal of poorly positioned and/or proud metallic suture anchors after Bankart repair. However, further clinical reports on larger series are warranted to document the efficacy of this procedure in selected cases.

Keywords

Arthroscopy, Shoulder, Surgery, Bankart Repair, Complication

1. Introduction

Arthroscopy of shoulder joint is been used commonly for diagnosis and treatment of glenohumeral joint pathologies. Intra-articular implants introduced in conjunction with arthroscopy have provided successful outcomes in terms of soft tissue reconstruction [1].

Arthroscopic Bankart repair surgery using suture anchors has become the most common surgery for management of post-traumatic anteroinferior instability of the shoulder joint. It mostly provides satisfactory outcomes; however, recurrence rates of instability may be more than expected rising up to rates of 35% - 40% especially in patients younger than 25 years of age. Moreover, the results seem to worsen during long-term follow-up [2] [3]. Since patients suffering from failed Bankart repair are generally young and active people, necessity for revision stabilization surgery often arises. In this purpose, open Bankart operation, revision arthroscopic Bankart operation or glenoid rim reconstruction with bone grafting can be used for restoration of joint stability [4] [5]. Nevertheless, the optimal technique for revision has not been well defined yet in the literature.

In parallel to the popularization of shoulder arthroscopy, there has been a remarkable increase in the frequency of complications associated with implants used for repair of rotator cuff or stabilization of shoulder joint. Suture anchors have been implemented frequently for fixation during soft tissue repair. However, poorly positioned suture anchors result in continuous pain, cartilage injury, restriction of motion and failure of reconstruction [6].

Male gender, young age, bony defects, hyperlaxity, and poor-quality joint capsule are among risk factors for failure of arthroscopic Bankart repair [7]. If 20% - 30% of the glenoid width is eroded, failure is more likely and unrecognised glenoid or humeral bony defects constitute common reasons for recurrence after Bankart repair [8] [9]. The variety and combination or interaction of soft tissue and bony pathologies complicate the identification of the appropriate method for revision surgery.

Arthroscopic shoulder surgery has been popularized recently in accordance with improvement of understanding of the complex anatomy and functioning of shoulder joint. However, in case that complications occur, there is mostly a lack of consensus on the method of management. Lack of guidance necessitates clarification for selection of the most appropriate method in the management of complications. The current study was carried out to present our outcomes for arthroscopic removal of poorly positioned and/or proud metallic suture anchors placed during Bankart repair in 14 consecutive cases.

2. Patients and Methods

2.1. Study Design

This retrospective study has been conducted in accordance with the principles of the Helsinki Declaration. Written informed consent was obtained from all subjects. Approval of local Institutional Review Board has been obtained priorly (February 2018).

A total of 14 patients who underwent open or arthroscopic Bankart repair with an initial presentation of traumatic shoulder instability between January 2010 and January 2017 and admitted to our center with complaints due to poorly positioned and/or proud metallic suture anchors were enrolled in our study. The data obtained from the patient records were information on the operative findings, surgical outcomes and complications.

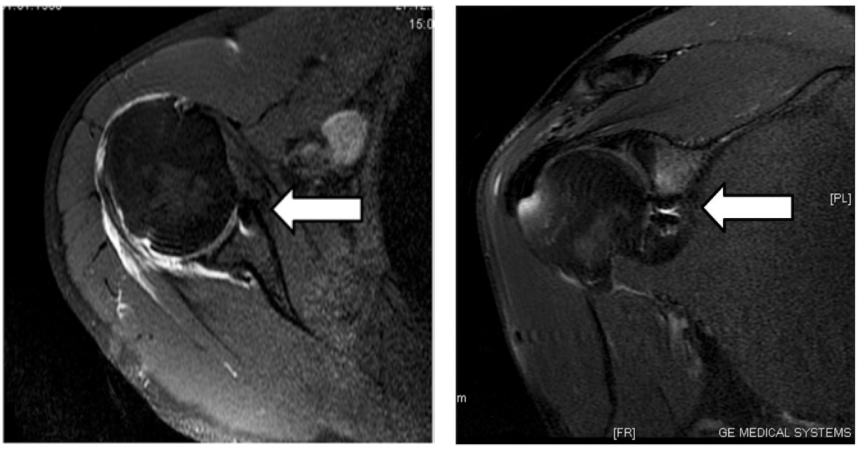
Inclusion criteria: The criteria for inclusion were 1) patients over 18 years, 2) the presence of traumatic shoulder instability in the initial admission, 3) Bankart repair open or arthroscopic with use of either open or arthroscopic techniques, 4) surgery performed by the same surgeon (O.G.) or under his direction.

Exclusion criteria: Exclusion criteria were 1) patients under 18 years, 2) using anchors other than metallic suture anchors.

2.2. Outcome Parameters

Pre- and intraoperative findings, surgical outcomes and complications were reviewed. Diagnosis of proud or poorly positioned suture anchors was established using magnetic resonance imaging (MRI) of shoulder in five cases, and with shoulder arthroscopy in nine patients (Figure 1(a) and Figure 1(b)).

Patients were evaluated the day before surgery and at last follow-up. Constant scores, and American Shoulder and Elbow Surgeons (ASES) scores were compared.



(a) (b)

Figure 1. (a) and (b) Magnetic resonance views demonstrating poorly positioned metallic anchors resulting in stiffness and pain of the shoulder joint (white arrow).

2.3. Statistical Analyses

Data were analyzed using the IBM Statistical Package for Social Sciences v21 (SPSS Inc., Chicago, IL, USA). Parametric tests were applied to data of normal distribution and non-parametric tests were applied to data of questionably normal distribution. The results for all items were expressed as mean ± SD, assessed within a 95% reliance and at a level of $p < 0.05$ significance.

3. Results

Demographic and initial surgical data of the patients are presented in Table 1. Eleven male and three female patients with an average age of 29.21 ± 5.78 (range, 20 to 42) were enrolled in the present study. The right side was affected in 9 (64.3%) cases and the left side in 5 (35.7%) cases. The dominant side was affected in 10 (71.4%) cases. Initially, eleven cases had undergone primary arthroscopic Bankart repair, while three patients had been operated with open Bankart repair. Metallic suture anchors have been placed during primary surgery in thirteen patients and during revision surgery in one patient. Mean number of metallic suture anchors used in the initial surgery was 2.36 ± 0.50 (range, 2 to 3). The main complaint was pain in 9 (64.3%) cases, metallic clicking sound in 8 (57.1%) cases, recurrent dislocation in 5 (35.7%) cases, and stiffness in 2 (14.3%) cases. Mean duration of the complaints was 5.57 ± 2.31 (range, 2 to 9) months. Metallic suture anchors aided the confirmation of diagnosis by radiological methods in 5 cases; however, it must not be neglected that confirmation of diagnosis required arthroscopy in 9 cases.

Revision Bankart repair was performed arthroscopically in all patients. Findings of revision surgery are presented in Table 2. In all cases, arthroscopic removal of poorly positioned suture anchors could be accomplished successfully. Suture anchors were found to be extending from the cartilaginous surface of the shoulder joint. In the patient whose poor positioning of the anchor occurred during revision surgery, removal of anchor was carried out using screwdriver. In eight cases, expulsion of anchors by rotation was managed using acutenaculum after dissection from surrounding tissues. In two cases, the suture anchor was taken out after dilatation of its periphery with a 6.5 mm mosaic plastic cannula used for autologous osteochondral transfer system (Figure 2(a) and Figure 2(b)) [10]. Grafting was not performed since the bare area was beyond the ventral part of the joint. Eleven cases had undergone primary arthroscopic Bankart repair, while three patients had been operated with open Bankart repair and the average operation time was 5.5 (range, 2 to 9) months. The mean follow-up period was 40.4 months, ranging from 18 to 64 months.

The preoperative Constant and ASES scores were 68.43 ± 7.05 and 38.3 ± 19.4, respectively. Postoperatively, the scores were 89.64 ± 5.39 and 89.07 ± 3.89, respectively ($p < 0.01$) (Table 3).

4. Discussion

The aim of the present study was to present our results in arthroscopic

Table 1. Demographic and initial surgical data of the patients.

No	Gender	Age (years)	Dominancy	Side	Previous Surgical Technique	Number of metallic anchors in the initial surgery	Complaints	Duration of complaint (month)	Anchor Problem
1	Male	20	Dominant	R	Arthroscopic	3	Pain Stiffness	2	Proud anchor
2	Female	42	Non-dominant	L	Arthroscopic	2	Pain Metallic clicking sound	8	Proud anchor
3	Male	35	Non-dominant	R	Arthroscopic	3	Pain Metallic clicking sound	9	Proud anchor
4	Female	30	Dominant	R	Arthroscopic	2	Recurrent dislocation	4	Poorl positioned anchor
5	Male	24	Dominant	L	Arthroscopic	2	Recurrent dislocation	5	Poorly positioned anchor
6	Male	26	Dominant	R	Open	2	Pain Metallic clicking sound	3	Proud anchor
7	Male	28	Dominant	R	Arthroscopic	3	Pain Metallic clicking sound	5	Proud anchor
8	Male	30	Non-dominant	L	Open	2	Pain Metallic clicking sound	6	Proud anchor
9	Female	33	Non-dominant	L	Arthroscopic	2	Recurrent dislocation	7	Poorly positioned anchor
10	Male	31	Dominant	L	Open	2	Pain Metallic clicking sound	8	Proud anchor
11	Male	20	Dominant	R	Arthroscopic	2	Pain Metallic clicking sound Stiffness	9	Proud anchor
12	Male	30	Dominant	R	Arthroscopic	3	Recurrent dislocation	5	Poorly positioned anchor
13	Male	32	Dominant	R	Arthroscopic	3	Pain Metallic clicking sound	4	Proud anchor
14	Male	28	Dominant	R	Arthroscopic	2	Recurrent dislocation	3	Poorly positioned anchor

management of poorly positioned and/or proud metallic suture anchors applied during or after Bankart repair. Our results indicate that arthroscopy seems to be a safe and effective alternative in the surgical management of these patients.

Table 2. Findings of revision surgery.

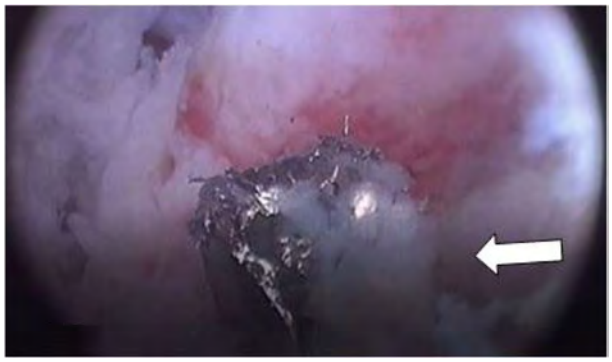
No	Anchor's position in the glenoid	Anchor's position (o'clock)	Surgical procedure in revision	Method of anchor removal	Number of anchors removed	Presence of intraarticular chondral destruction Humerus/glenoid	Follow-up (months)
1	Glenoid rim	3	Removal of anchor	Screwdriver	1	No	25
2	Glenoid 5 mm medial	3	Removal of anchor	Screwdriver	1	Yes Humerus	56
3	Glenoid 10 mm medial	3 - 5	Removal of anchor	Autologous osteochondral transfer system	2	Yes Glenoid	60
4	Glenoid rim	4	Removal of anchor Revision Bankart repair	Screwdriver	1	Yes Humerus	34
5	Glenoid rim	4	Removal of anchor Revision Bankart repair	Screwdriver	1	Yes Humerus	48
6	Glenoid 8 mm medial	3	Removal of anchor	Screwdriver	1	Yes Humerus	24
7	Glenoid 10 mm medial	3	Removal of anchor	Screwdriver	1	Yes Glenoid	18
8	Glenoid rim	3	Removal of anchor	Screwdriver	1	Yes Humerus	64
9	Glenoid rim	4	Removal of anchor Revision Bankart repair	Autologous osteochondral transfer system	1	Yes Humerus	48
10	Glenoid 5 mm medial	3	Removal of anchor	Screwdriver	1	No	32
11	Glenoid 5 mm medial	3	Removal of anchor	Screwdriver	1	No	36
12	Glenoid rim	4	Removal of anchor	Autologous osteochondral transfer system	1	Yes Humerus	25
13	Glenoid rim	3	Removal of anchor	Screwdriver	1	Yes Humerus	56
14	Glenoid 10 mm medial	5	Removal of anchor	Autologous osteochondral transfer system	1	Yes Humerus	60

Table 3. Clinical scores in preoperative and postoperative periods.

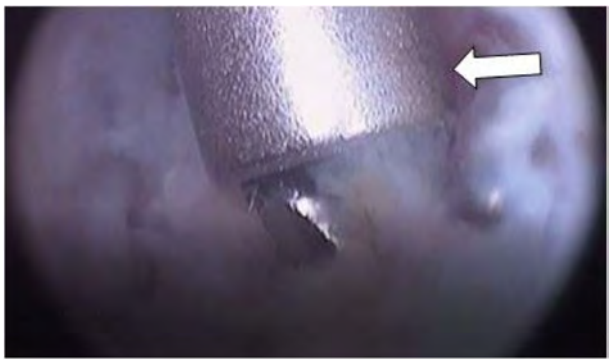
	Preoperatively mean ± SD (range)	Postoperatively mean ± SD (range)	*P* value
Constant score	68.43 ± 7.05 (59 - 83)	89.64 ± 5.39 (82 - 98)	<0.01
ASES score	64.00 ± 7.14 (55 - 81)	89.07 ± 3.89 (86 - 98)	<0.01

ASES = American Shoulder and Elbow Society.

Reported rates of complications vary between 4.6% - 10.6% [11]. Careful selection of patients, understanding and adherence of indications, good knowledge of anatomy and appropriate patient positioning are useful measures that may aid in reduction of complication rates [12]. In case complications occur, there is



(a)



(b)

Figure 2. (a) Arthroscopic appearance of poorly positioned suture anchor (white arrow); (b) Arthroscopic appearance of peripheral dilatation of poorly positioned suture anchor with autologous osteochondral transfer system canula (white arrow).

mostly a lack of consensus on the method of management. Lack of guidance necessitates clarification for selection of the most appropriate method in the management of complications. Choice of metal or radiolucent implants can determine the follow-up of the position of implants in the postoperative period [11] [12]. In our series, metal anchors aided the confirmation of diagnosis by radiological methods in 3 cases, but it must not be neglected that confirmation of diagnosis required arthroscopy in 8 cases.

Zuckerman *et al.* reviewed 37 cases with glenohumeral joint complications after open surgery and revealed that ten patients had erosive changes in the humeral head or the glenoid cavity associated with the incorrect placement of the fixture [13]. Kaar *et al.* studied 8 cases with complications after open surgery in which metals utureanchors were used and showed that 3 cases developed serious joint damage caused by a loose or exposed metal anchor [14]. Ejnisman *et al.* studied eight cases with complications due to the use of anchors in open and arthroscopic surgeries and showed that all cases had chondral injuries of the humeral head and 80% had chondral injuries of the glenoid cavity [15]. Our study shows complications with the use of metal anchors. Of the 14 patients total, 11 had glenohumeral chondral damage in various grades.

Koss *et al.* observed a case in which there was increasing pain and crepitus a few weeks after open stabilization of a Bankart lesion [16]. The symptoms were

more prominent in abduction and internal rotation of the arm. In our series, we found that the most common symptoms were pain and metallic clicking sound.

Rhee *et al.* reported performing the second surgery an average of 12 months after primary surgery [17]. Ejnisman *et al.* reported that only one (12.5%) of the eight patients was revised in the first six weeks, the remainder were revised after three months [15]. The data in the literature regarding the time of the revision are contradictory. In our study, the time between the first and second surgeries was 5.5 months.

Even though recurrence after arthroscopic surgery is still a challenge, new technology and improved surgical practice have yielded better functional results with less morbidity. On the other hand, a meta-analysis by Lenters *et al.* have shown that arthroscopic suture techniques were linked with increased risks of recurrent instability and dislocation compared to open technique [18]. Despite this report, arthroscopic repair was found to provide better functional scores than open surgical methods [6]. It must be remembered that arthroscopy offers a better diagnostic ability, facility to repair all accompanying lesions, dimunition of likelihood of stiffness, shorter time of surgery and less postoperative pain compared to open technique. Technical errors such as poor positioning of the anchors can lead to failure of arthroscopic Bankart repair even in patients with appropriate indications [19]. In addition to poor positioning of the anchors, insufficient number of suture anchors or inappropriate depth of knotless anchors may be linked with recurrence [19]. Our case series denotes that poor positioning of suture anchors is not a rare entity that must be kept in mind while revisiting risk factors for failure of arthroscopic surgery. Identification of factors underlying failure accurately is mandatory for establishment of correct management strategy.

Regarding clinical results, we observed that the arthroscopic Bankart repair showed a significant improvement in outcome scores. The Constant and ASES scores increased from 68.43 ± 7.05 and 38.3 ± 19.4, respectively, in the preoperative evaluation to89.64 ± 5.39 and 89.07 ± 3.89, respectively at the postoperative follow-up ($p < 0.01$).

Limitation of our study includes the retrospective design and relatively small number of patients in our series. This is high-volume surgeon operating in a high-volume hospital, and studies have shown that these 2 factors contribute to improved clinical outcomes. It is likely that surgeon and hospital volume affected both the technical and operational efficiency and lowered our complication rates. As a result, relatively small number of patients was included in our series. In addition, some details of history and factors that may influence the outcome may not be completely documented. Due to these restrictions, associations should be interpreted with caution. Further prospective, controlled trials on larger series are necessary for making more precise interpretations.

5. Conclusion

In conclusion, arthroscopic surgery may yield an effective surgical option for

removal of poorly positioned and/or proud metallic suture anchors applied during or after Bankart repair. However, further clinical reports on larger series are warranted to document the efficacy of this procedure in selected cases.

Acknowledgements

Special thanks to Harun Mutlu, MD because of his contributions for verbal presentation in 8th Turkish Shoulder and Elbow Surgery Congress.

Financial

No financial support was received for this paper.

Conflicts of Interest

The authors declare no competing interests.

References

[1] Flinkkilä, T. and Sirniö, K. (2015) Open Latarjet Procedure for Failed Arthroscopic Bankart Repair. *Orthopaedics & Traumatology*: *Surgery & Research*, **101**, 35-38. https://doi.org/10.1016/j.otsr.2014.11.005

[2] Flinkkilä, T., Hyvönen, P., Ohtonen, P. and Leppilahti, J. (2010) Arthroscopic Bankart Repair: Results and Risk Factors of Recurrence of Instability. *Knee Surgery*, *Sports Traumatology*, *Arthroscopy*, **18**, 1752-1758. https://doi.org/10.1007/s00167-010-1105-5

[3] Voos, J.E., Livermore, R.W., Feeley, B.T., Altchek, D.W., Williams, R.J., Warren, R.F., *et al.* (2010) Prospective Evaluation of Arthroscopic Bankart Repairs for Anterior Instability. *The American Journal of Sports Medicine*, **38**, 302-307. https://doi.org/10.1177/0363546509348049

[4] Cho, N.S., Woong, J., Lee, B.G. and Rhee, Y.G. (2009) Revision Open Bankart Surgery after Arthroscopic Repair for Traumatic Anterior Shoulder Instability. *The American Journal of Sports Medicine*, **37**, 2158-2164. https://doi.org/10.1177/0363546509339015

[5] Abouali, J.A.K., Hatzatoni, K., Holtby, R., Veilette, C. and Theodoropoulos, J. (2013) Revision Arthroscopic Bankart Repair. *Arthroscopy*, **29**, 1572-1578. https://doi.org/10.1016/j.arthro.2013.04.017

[6] Noud, P.H. and Esch, J. (2013) Complications of Arthroscopic Shoulder Surgery. *Sports Medicine and Arthroscopy Review*, **21**, 89-96. https://doi.org/10.1097/JSA.0b013e31829006f0

[7] Randelli, P., Ragone, V., Carminati, S. and Cabiza, P. (2012) Risk Factors for Recurrence after Bankart Repair. A Systematic Review. *Knee Surgery*, *Sports Traumatology*, *Arthroscopy*, **20**, 2129-2138. https://doi.org/10.1007/s00167-012-2140-1

[8] Sommaire, C., Penz, C., Clavert, P., Klouche, S., Hardy, P. and Kempf, J.F. (2012) Recurrence after Arthroscopic Bankart Repair: Is Quantitative Radiological Analysis of Bone Loss of Any Predictive Value. *Orthopaedics & Traumatology*: *Surgery & Research*, **98**, 514-519. https://doi.org/10.1016/j.otsr.2012.03.015

[9] Burkhart, S.S. and De Beer, J.F. (2000) Traumatic Glenohumeral Bone Defects and Their Relationship to Failure of Arthroscopic Bankart Repairs: Significance of the Inverted Pear Glenoid and the Humeral Engaging Hill-Sachs Lesion. *Arthroscopy*, **16**, 677-694. https://doi.org/10.1053/jars.2000.17715

[10] Grutter, P.W., McFarland, E.G., Zikria, B.A., Dai, Z. and Petersen, S.A. (2010) Techniques for Suture Anchor Removal in Shoulder Surgery. *The American Journal of Sports Medicine*, **38**, 1706-1710. https://doi.org/10.1177/0363546510372794

[11] Marecek, G.S. and Saltzman, M.D. (2010) Complications in Shoulder Arthroscopy. *Orthopedics*, **33**, 492-497. https://doi.org/10.3928/01477447-20100526-15

[12] Moen, T.C., Rudolph, G.H., Caswell, K., Espinoza, C., Burkhead, W.Z. and Krishnan, S.G. (2014) Complications of Shoulder Arthroscopy. *The Journal of the American Academy of Orthopaedic Surgeons*, **22**, 410-419. https://doi.org/10.5435/JAAOS-22-07-410

[13] Zuckerman, J.D. and Matsen, F.A. 3rd (1984) Complications about the Glenohumeral Joint Related to the Use Screws and Staples. *The Journal of Bone and Joint Surgery. American Volume*, **66**, 175-180. https://doi.org/10.2106/00004623-198466020-00003

[14] Kaar, T.K., Schenck, R.C., Wirth, M.A. and Rockwood, C.A. (2001) Complications of Metallic Suture Anchors in Shoulder Surgery: A Report of 8 Cases. *Arthroscopy*, **17**, 31-37. https://doi.org/10.1053/jars.2001.18246

[15] Ejnisman, B., Andreoli, C.V., Pochini, A.C., Monteiro, G.C., Feloppa, F. and Cohen, M. (2006) Artropatiaglenoumeralpós-tratamento de lesõeslabiais com implantes-metálicos. *Revista Brasileira de Ortopedia*, **41**, 167-172.

[16] Koss, S., Richmond, J.C. and Woodward, J.R. (1997) Two to Five-Year Follow up of Arthroscopic Bankart Reconstruction Using a Suture Anchor Technique. *The American Journal of Sports Medicine*, **26**, 809-812. https://doi.org/10.1177/036354659702500613

[17] Rhee, Y.G., Lee, D.H., Chun, I.H. and Bae, S.C. (2004) Glenohumeral Arthropathy after Arthroscopic Anterior Shoulder Stabilization. *Arthroscopy*, **20**, 402-406. https://doi.org/10.1016/j.arthro.2004.01.027

[18] Lenters, T.R., Franta, A.K., Wolf, F.M., Leopold, S.S. and Matsen, F.A. 3rd (2007) Arthroscopic Compared with Open Repairs for Recurrent Anterior Shoulder Instability. A Systematic Review and Meta-Analysis of the Literature. *The Journal of Bone and Joint Surgery. American Volume*, **89**, 244-254. https://doi.org/10.2106/00004623-200702000-00003

[19] Law, B.K., Yung, P.S., Ho, E.P., Chang, J.J. and Chan, K.M. (2008) The Surgical Outcome of Immediate Arthroscopic Bankart Repair for First Time Anterior Shoulder Dislocation in Young Active Patients. *Knee Surgery, Sports Traumatology, Arthroscopy*, **16**, 188-193. https://doi.org/10.1007/s00167-007-0453-2

International Journal of Clinical Medicine, 2019, 10, 553-564
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Respiratory Disorders in Acromegalic Patients

Valeria Mercuri[1], Tullia Villani[2], Denise Costa[1], Michela Mordenti[2], Tania D'Amico[1], Paolo Palange[2], Patrizia Gargiulo[1]

[1]Department of Experimental Medicine, Endocrinology-Pituitary Disease, "Sapienza" University of Rome, Rome, Italy
[2]Department of Public Health and Infectious Diseases, "Sapienza" University of Rome, Rome, Italy
Email: v.mercuri79@gmail.com, tulliavillani85@yahoo.it, denise.costa@libero.it, michelamordenti@tiscali.it, damico.ta@gmail.com, paolo.palange@uniroma1.it, patrizia.gargiulo@uniroma1.it

How to cite this paper: Mercuri, V., Villani, T., Costa, D., Mordenti, M., D'Amico, T., Palange, P. and Gargiulo, P. (2019) Paper Title. *International Journal of Clinical Medicine*, **10**, 553-564.
https://doi.org/10.4236/ijcm.2019.1010045

Received: September 16, 2019
Accepted: October 20, 2019
Published: October 23, 2019

Abstract

Purpose: To evaluate the prevalence and clinical performance of obstructive sleep apnoea syndrome (OSAS) in acromegalic patients, focusing on the possible correlation between alterations of pulmonary microcirculation and patient's clinical and hormonal parameters. **Methods:** We enrolled 22 acromegalic patients with apnea hypopnea index (AHI) ≥ 5 if symptomatic, or ≥15 in the absence of sleep related symptoms. Patients underwent the following evaluations: GH and IGF-1 serum levels, arterial blood gas test, spirometry, carbon monoxide diffusing capacity (DLCO), home-based cardiorespiratory sleep and pulmonary function test. **Results:** The prevalence of OSAS was 66.6%. Patients with a severe form of OSAS appeared to be affected from acromegaly for more years than those with a lower severity of disease and those with acromegaly duration > 10 years had significantly higher levels of glycated hemoglobin. We observed a positive correlation between GH level at diagnosis and DLCO value, showing that acromegalic patients have an increase in static and dynamic respiratory volumes and alveolar-capillary exchange surface compared to the general population. We also observed a positive correlation between baseline serum IGF-1 level and DLCO/VA and between years of disease and DLCO/VA, showing the association of increased lung volume and increase of the exchange surface. **Conclusions:** The results of this study add new information on lung volume and alveolar gas exchange in acromegaly. Our findings highlight the role of SSA as therapy with positive impact on complication and comorbidities of acromegaly.

Keywords

Acromegaly, Obstructive Sleep Apnoea Syndrome, Growth Hormone, Insulin-Like Growth Factor 1, Carbon Monoxide Diffusing Capacity

1. Introduction

Acromegaly is a severe disease characterized by hypersecretion of growth hormone (GH), which induces the synthesis of peripheral insulin-like growth factor 1 (IGF-1) after the closure of the epiphyseal bone. Acromegaly is usually the result of a GH-secreting pituitary adenoma, which results in anatomical changes and metabolic dysfunction. In rare cases, the disease is associated with excessive production of GH-releasing hormone (GHRH), familial syndromes, including multiple endocrine neoplasia type 1, McCune-Albright syndrome, familial acromegaly, and Carney's syndrome. Extrapituitary ectopic hypersecretion of GH has been reported in isolated cases of pancreatic islet-cell tumors or lymphoma. Clinical manifestations of acromegaly range from subtle signs of acral overgrowth, soft tissue swelling, arthralgias, jaw prognathism, fasting hyperglycemia, and hyperhidrosis to florid osteo-arthritis, frontal bone bossing, diabetes mellitus, hypertension, respiratory and heart failure [1] [2].

In recent years, there has been mounting interest in respiratory disorders during sleep in patients with acromegaly. In fact the anatomical changes commonly occurring in patients with acromegaly, such as facial soft-tissue thickening (especially of the palate and uvula), may induce the onset of the obstructive sleep apnoea syndrome (OSAS), due to hypercollapsibility of the posterior and lateral hypopharyngeal walls, as well as to hypertrophy of the tongue [3].

Nocturnal breathing disorders affect the majority of patients with acromegaly. Sleep apnoea syndrome (SAS), diagnosed by polysomnography, has been reported in about 70% of patients with active acromegaly disease; the frequency, however, ranges from 45% to 80% [3] [4]. SAS can be central or mixed, but it is obstructive in most cases.

OSAS is a highly prevalent sleep disorder characterized by a transient cessation in breathing (apnea) or a significant reduction in breathing amplitude (hypopnea), due to recurrent episodes of upper airway obstruction and subsequent recurrent arousal during sleep. This syndrome is characterized by daytime sleepiness, non-restorative sleep, fatigue, insomnia, loud snoring, witnessed breathing interruptions, morning headaches, and recurrent awakenings due to gasping or choking in the presence of at least five obstructive respiratory events (apneas, hypopneas, or respiratory effort-related arousals) per hour of sleep. The presence of 15 or more obstructive respiratory events per hour of sleep in absence of sleep-related symptoms is also sufficient for a diagnosis of OSAS, due to the greater association of this severe degree of obstruction with important complications, such as increased cardiovascular and metabolic disease risk [5]. Early markers of pulmonary damage may however have a great importance in the diagnosis of OSAS, in order to institute prompt treatment.

The principal aim of this study was to further evaluate the prevalence and secondly the clinical performance of OSAS in relation to duration of acromegalic disease. Moreover, we assessed the possible correlation between the alterations in gaseous exchanges at the level of pulmonary microcirculation and the clinical

and hormonal parameters, to identify possible early markers of pulmonary performance damage.

2. Patients and Methods

2.1. Patients

Population: Patients referring to the Metabolic Emergencies-Pituitary Disease Unit of the Department of Experimental Medicine, Policlinico Umberto I-"Sapienza" University of Rome, were evaluated in this study. The local Ethical Committee approved the study design, and all patients signed an informed consent to the use of their data for research purposes.

Inclusion criteria: patients with the diagnosis of acromegaly (in agreement to consensus on the medical treatment of acromegaly) (November 2014) [6].

Exclusion criteria: Patients affected from major systemic diseases such as malignant cancer, immune disorders, acute cardiovascular and cerebrovascular diseases or other disorders correlated with apnea (e.g. hypothyroidism), or those with intake of psychotropic drugs, alcoholism, smoking and other abuses, were excluded. All patients were biochemically targeted for any other hormone deficiency.

Baseline characteristics of study population are described in **Table 1**.

2.2. Diagnosis of Acromegaly

The diagnosis of acromegaly was performed on the bases of the latest available guidelines, which, in patients with elevated or equivocal serum IGF-1 concentrations, recommend confirmation of the diagnosis by finding lack of suppression of GH to <0.4 μg/l, following documented hyperglycemia during an oral glucose load (2 hours after 75 g of oral glucose) [6] [7] [8].

Table 1. Clinical data.

Study population	
Number of patients (11 M and 11 F)	22
Age (years)	58.4 ± 19.5
Mean DD	12.9 ± 8.9 years
	<10 years in 10/22 patients
	>10 years in 12/22 patients
Incidence of OSAS	66.6% (15/22)
Mild grade	40% (6/15)
Moderate grade	26.6% (4/15)
Severe grade	33.3% (5/15)
HbA1c (%) in OSAS patients with DD > 10 years	6.2 ± 1.4
HbA1c (%) in OSAS patients with DD < 10 years	5.1 ± 0.5

DD: Duration of disease from diagnosis; HbA1c: glycated hemoglobin; OSAS: Obstructive Sleep Apnoea Syndrome.

Biochemical control of disease was defined on the basis of the determination of IGF-1, age-related and expressed in ng/ml [6]. GH concentrations were not used for the evaluation of the biochemical control of disease since seven patients were treated with pegvisomant [6].

2.3. IGF-1 and GH Serum Assay

The determination of GH and IGF-1 run with chemiluminescent immunometric assay (SIEMENS Immunolite 2000) was performed at the Laboratory of Clinical Pathology of the Policlinico Umberto I.

The patients were referred to our Sleep Disorders Unit and underwent blood sampling (for the assessment of GH and IGF-1 serum levels), arterial blood gas test (ABG), spirometry, carbon monoxide diffusing capacity (DLCO) and home-based cardiorespiratory sleep. The study protocol has been defined in agreement with the ethical principles of the Helsinki Declaration. All patients gave written informed consent for their participation in the study.

2.4. Home-Based Cardiorespiratory Sleep Study

A home-based cardiorespiratory sleep study was performed by using a SOMNO screen device (SOMNO medics-GmbH, Randersacker, Germany). The study recorded the following parameters: chest and abdominal movements, nasal flow, body position, oxygen saturation, snoring, and heart rate. Daytime sleepiness was assessed using the Epworth Sleepiness Scale (ESS) [9], and symptoms such as daytime sleepiness, non-restorative sleep, fatigue, insomnia, snoring, witnessed breathing interruptions, morning headaches, and recurrent awakenings due to gasping or choking were recorded. Cardiorespiratory analysis was examined according to the American Academy of Sleep Medicine (AASM) 2007 guidelines [10].

Apnea was defined as the cessation of airflow for a period ≥ 10 s, and hypopnea was defined either as the reduction of at least 30% of the airflow associated with oxygen desaturation ≥ 4% or as a ≥50% airflow reduction with a ≥3% oxygen desaturation for ≥10 s. From each recording, we assessed the Apnea Hypopnea Index (AHI; number of apneas and hypopneas per hour), percentage of total sleep time passed with an oxygen saturation < 90% (T < 90%) and the Oxygen Desaturation Index (ODI; number of oxygen desaturations per hour), as parameters of disease severity. Inclusion criteria were AHI ≥ 5 in symptomatic patients or AHI ≥ 15 in the absence of sleep related symptoms.

2.5. Pulmonary Function Test

Pulmonary function data were performed by standardized techniques using an automated pulmonary function testing system (COSMED PFT, Pavona Italy). The following tests were collected and standardized as percentages of predicted normal values: spirometry, nitrogen washout and single-breath diffusing capacity (DLCO) [10] [11].

3. Statistical Analysis

All data were tested to verify if assumptions of normality were true. The assumptions of normality could not be assumed within the data set particularly due to the small sample size. Therefore, nonparametric tests were used. Continuous data were compared using Wilcoxon rank-sum test. The Mann-Whitney U test or the Kruskal-Wallis was used to assess differences between the two groups (target and no target patients) and three groups according to OSAS severity (mild, moderate and severe). Spearman's rank order correlation coefficient (r) was used to test associations between numerical variables. Data are reported as mean ± standard error (SE). P values < 0.05 were considered significant. Multivariate analysis was applied for the estimation of the relationships between polysomnography variables, spirometry data and GH and IGF-1 serum levels. A commercial software (SPSS version 20.0) was used for statistical analysis.

4. Results

We selected 22 acromegalic patients, out of a total of 180 attending our Center, on the basis of symptoms or signs of respiratory disorders, evaluating among these the presence or absence of OSAS and the degree of severity.

The study included 22 acromegalic patients (11 males and 11 females). Table 1 shows baseline characteristics of study population.

At enrolment, the mean duration of disease from diagnosis (DD) was 12.9 ± 8.9 years (>10 years in 12/22 subjects). All patients presented biochemical control of acromegalic disease, with normal age-adjusted levels of IGF-1.

The prevalence of OSAS was 66.6% (15/22 of cases).

In more details, 40% of them (6/15) showed a mild OSAS grade (AHI 10.6 ± 2 events per hour), 26.6% (4/15) a moderate grade (AHI 19.9 ± 4.4 events per hour) and 33.3% (5/15) a severe OSAS (AHI 41.1 ± 9.9 events per hour) (Table 1).

When stratifying patients according to OSAS severity (mild, moderate, severe), we observed that severe OSAS patients appear to have been affected by acromegaly for several years (determined illness plus suspected disease) (20.4 ± 9.4 years vs 14.7 ± 5.2 vs 34.1 ± 17.5; p = 0.05).

Significantly higher values of glycated hemoglobin (HbA1c) were found in OSAS patients with acromegaly duration > 10 years compared with those with <10 years of disease (6.2 ± 1.4 vs 5.1 ± 0.5; p = 0.01) (Table 1).

Spirometry data of our patients showed the following values: forced vital capacity (FVC) (L) 4.32 ± 1.6, FVC % 116.3 ± 21.6, forced expiratory volume in 1 second (FEV1) (L) 3.3 ± 1.7, FEV1 % 110.4 ± 21, FEV1/FVC % 77.67 ± 7.7, total lung capacity (TLC) % 105.2 ± 27.9, DLCO % 100 ± 27.9.

We disclosed a positive correlation between serum GH level at the time of the acromegaly diagnosis and DLCO value (110.1 ± 27.9) (p = 0.04). A further positive correlation was demonstrated between baseline serum IGF-1 level and

DLCO/alveolar volume (VA) value and between years disease (illness plus suspected disease) and DLCO/VA (103.5 ± 17.9; p = 0.04).

A negative correlation between the average of the last three determinations of serum IGF-1 levels, the values of serum bicarbonates (HCO^{3-}) (26.5 ± 2.1; p = 0.04) and carbon dioxide (pCO_2) (40.6 ± 4.3; p = 0.019) was reported in all patients included in the study.

Lastly, in target patients the average of the last three determinations of serum IGF-1 level correlated negatively with the partial pressure of pCO_2, determined at the time of enrolment with arterial blood gas test (41 ± 4.1 mmHg; p = 0.01).

5. Discussion

OSAS is a common disorder characterized by recurrent episodes of apnea or hypopnea, due to total or partial pharyngeal collapse and temporary upper airway obstruction during sleep, resulting in frequent episodes of hypoxemia and hypercapnia. Some endocrine and metabolic disorders (obesity, acromegaly, hypothyroidism, etc.) are associated with a high frequency of OSAS, and treatment of the underlying endocrine disorder can improve sleep disorders [12] [13] [14] [15].

Two hypothesis was proposed to explain the high prevalence of OSAS in acromegaly: 1) the influence of abnormal levels of pituitary hormones on impaired respiratory control; 2) the anatomical changes in the upper airways body changes are derived especially from skeletal growth and soft tissue enlargement, which is subtle in the early stage of disease [16] [17].

Facial changes include anatomical disorders (large lips and noses, macroglossia, mandibular overgrowth with prognathism, maxillary widening …). Dostalova S. *et al.* argue that these anatomical disorders are not sufficient to cause OSAS, while soft tissue thickening is the more evident contributor to OSAS onset [18].

In several prospective or retrospective studies, the prevalence of OSAS in acromegaly is around 69% [19], vs 66.6% in ours. In more details, 40% affected by mild OSAS, 26.6% by moderate and 33.3% by severe. Roemmler J. *et al.* showed a positive correlation between respiratory disturbance index (RDI) and acromegalic disease activity, but not with the duration of the disease [20]. Another study reported a positive correlation between the severity of sleep-disordered breathing and duration of acromegaly in patients with active disease. Stratifying OSAS patients according to disease severity we observed that severe OSAS patients appear to be affected from acromegaly for more years than those with a lower severity of disease [21] [22].

Previous studies suggest that enhanced lung growth in acromegalic patients is associated with either a normal or above normal pulmonary transfer factor in pulmonary function test. Brody *et al.* suggest either alveolar hypertrophy or hyperplasia as the mechanism for lung growth in this condition. In fact, normal postnatal lung growth occurs in two major stages: the former, primarily asso-

ciated with an increase in alveolar numbers, the latter correlated with an increase of alveolar size. Growth hormone excess resulted in the formation of new alveoli and new surface for gas exchange, therefore diffusing capacity (DL) should be increased in the acromegalic subjects. On the other hand, normal DL suggests that lung growth was associated with increased alveolar size; the increased alveolar volume or size of the pulmonary capillary bed was offset by the effects of increased cell size and interstitial tissue in impeding diffusion [23].

In our study we found a positive correlation between serum GH level at acromegaly diagnosis and DLCO. Our results showed that the static and dynamic respiratory volumes and alveolar-capillary exchange surface are increased if compared with the general population (adjusted for gender, age and weight) as shown by TLC%, FEV1 (in liters and %), FVC (in liters and %) and DLCO%.

Another hormone involved in tissue growth is IGF-1, which is generally accepted as a central mediator of metabolic, endocrine, and anabolic effects of GH. Moreover, an association of circulating IGF-I levels and effects on muscular strength of rehabilitation programs in patients with chronic obstructive pulmonary disease has been described [24] [25].

Glaser *et al.* reported a positive association between IGF-1 serum levels and higher lung volumes in men of all ages and women older than 50 years. IGF-1 serum values or increasing lung volumes were not associated with increased strength of respiratory muscles [26].

To this end, our results show positive correlation between baseline serum IGF-1 levels, DLCO/VA value and between years of disease (illness plus suspected disease) and DLCO/VA showing the association of increased lung volume and increase of the exchange surface (alveolar hypertrophy or hyperplasia).

The duration of the disease, as expected, plays an important role on the alterations of respiratory function in acromegalic patient. In fact, our data show that longer duration of illness is correlated with increased incidence of OSAS.

IGF-1 exerts multiple physiological effects on the vascular system, including proliferative, hypertrophic, survival, vasomotor and metabolic effects. The expression of IGF-1, IGF-1R, and IGFBPs in the blood vessels is regulated by multiple factors, including growth factors, cytokines, lipoproteins, reactive oxygen species and hemodynamic forces. The close interaction between IGF-1 and other growth factors system at the level of receptor-ligand and at the level of post-receptor signaling pathways has important implications in the understanding of the involvement of IGF-1 system in vascular diseases. Although recent studies have indicated that IGF-1 has a strong effect on angiogenesis [27] [28], it has been reported that IGF-1 can induce angiogenesis in skeletal muscle and brain tissue. This mechanism may also be present in the lung, thus justifying the increase in DLCO in patients with OSAS by increased alveolar capillarization.

Therefore, some authors demonstrated in male volunteers with high IGF-1 levels ratio values higher FEV1 and FVC values [26].

Increase in lung size has been described in acromegalic patients, but data on respiratory muscle function and control of breathing are relatively scant. Some

patients with acromegaly show decreased respiratory muscle strength, although the magnitude of this change has not been significantly correlated with the increase in lung volume, or with measurements of arterial $PaCO_2$ and PaO_2) [29]. Peripheral (muscular) factors appear to modulate a normal central motor output to give a more rapid pattern of breathing. Varying levels of hypoxemia with increased alveolar-arterial oxygen gradients have been described in patients with acromegaly, thereby suggesting disturbance of the ventilation/perfusion relationship [30].

As a further support of a possible alteration of the ventilation/perfusion and of a more rapid pattern of breathing, our data showed a negative correlation between the average of the last three determinations of serum IGF-1 level and the concentration of serum bicarbonates (HCO^{3-}) and CO. Last, in target patients the average of the last three determinations of serum IGF-1 level correlates negatively with the partial pressure of carbon dioxide, determined at enrolment with arterial blood gas test. Given the trend of bicarbonate and arterial carbon dioxide values in relation to hormonal IGF-1 values, we could speculate that in acromegalic patients the anatomical and functional changes of the lung can lead to a pattern of hyperventilation, despite the present diagnosis of OSAS. Different studies report the effects of GH/IGF-1 administration on the renal and systemic regulation of acid-base homeostasis.

The finding that GH administration results in markedly increased rates of renal hydrogen secretion and sodium chloride reabsorption, sufficient to cause these effects: 1) an increase in net acid excretion despite reclamation of an increased filtered load of bicarbonate; 2) an expanded extracellular fluid volume, as evidenced by weight gain and sodium retention despite an increased filtered sodium load, cannot be explained by the effects of enhanced mineral or glucocorticoid activities which decreased in response to GH [31] [32].

In contrast to these data we found in the acromegalic group bicarbonate values not very high compared with hormonal values, likely because such data were biased by the use of specific drugs such as somatostatin analogues, bromocriptine and receptor antagonists for the hormone growth or because the patients had been subjected to surgical intervention.

6. Conclusions

Our data are consistent with current literature on the prevalence, disease duration, body mass index (BMI), gender, neck circumference and Epworth Score of OSAS in patients with acromegaly, and do not show a statistically significant correlation with disease control. They also add new information on lung volumes and the alveolar gas exchange.

In relation to the therapy in acromegalic patients with OSAS, there is an unambiguous suggestion. Our study, however, has highlighted the role of the somatostatin analogues (SSAs) as therapy with a positive impact on complications and comorbidities of acromegalic disease. In the respiratory system, in fact, these molecules reduce the tissue turgidity and the sensitivity of chemoreceptors to

hypoxia by improving breathing. Moreover, the effectiveness of SSAs as antinociceptive and analgesic on headache symptoms is underlined, further to the already known effects of shrinkage of pituitary adenoma and biochemical control of disease. For this reason, we are evaluating for a subsequent work the comparison of respiratory parameters in naively acromegalic patients versus patients in therapy with SSAs.

As recommended since the guidelines of Versailles in 2003 [7], given the prevalence of OSAS in acromegaly and associated comorbidities, it is mandatory to perform a polysomnographic examination in all patients with acromegaly at diagnosis and during the follow-up. In fact, since 25% of the mortality is due to respiratory disorders, early diagnosis of OSAS not only improves life expectancy and quality of life of patients, but also contributes to the reduction of cardiovascular risk with considerable impact on health care costs.

Acknowledgements

Editorial assistance for the preparation of this manuscript was provided by Luca Giacomelli, PhD Ambra Corti, and Pascal Vignally, M.D. on behalf of Content Ed Net; this assistance was supported by Novartis.

Funding

No funding was received for the preparation of this manuscript.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent

Informed consent was obtained from all individual participants included in the study.

Conflicts of Interest

All the authors declare they have no conflict of interest.

References

[1] Melmed, S. (2006) Acromegaly. *The New England Journal of Medicine*, **355**, 2558-2573. https://doi.org/10.1056/NEJMra062453

[2] Davì, M.V. and Giustina, A. (2012) Sleep Apnea in Acromegaly: A Review on Prevalence, Pathogenetic Aspects and Treatment. *Expert Review of Endocrinology and Metabolism*, **7**, 55-62. https://doi.org/10.1586/eem.11.82

[3] Vannucci, L., Luciani, P., Gagliardi, E., Paiano, S., Duranti, R., *et al.* (2013) Assessment of Sleep Apnea Syndrome in Treated Acromegalic Patients and Correlation of Its Severity with Clinical and Laboratory Parameters. *Journal of Endocrinological*

Investigation, **36**, 237-242.

[4] Fatti, L.M., Scacchi, M., Pincelli, A.I., Lavezzi, E. and Cavagnini, F. (2001) Prevalence and Pathogenesis of Sleep Apnea and Lung Disease in Acromegaly. *Pituitary*, **4**, 259-262. https://doi.org/10.1023/A:1020702631793

[5] Berry, R.B., Budhiraja, R., Gottlieb, D.J., Gozal, D., Iber, C., *et al.* (2012) Rules for Scoring Respiratory Events in Sleep: Update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *Journal of Clinical Sleep Medicine*, **8**, 597-619. https://doi.org/10.5664/jcsm.2172

[6] Katznelson, L., Laws, E.R., Melmed, S., Molitch, M.E., Murad, M.H., *et al.* (2014) Acromegaly: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, **99**, 3933-3951. https://doi.org/10.1210/jc.2014-2700

[7] Giustina, A., Casanueva, F.F., Cavagnini, F., Chanson, P., Clemmons, D., *et al.* (2003) Diagnosis and Treatment of Acromegaly Complications. *Journal of Endocrinological Investigation*, **26**, 1242-1247. https://doi.org/10.1007/BF03349164

[8] Melmed, S., Casanueva, F.F., Klibanski, A., Bronstein, M.D., Chanson, P., *et al.* (2012) A Consensus on the Diagnosis and Treatment of Acromegaly Complications. *Pituitary*, **16**, 294-302. https://doi.org/10.1007/s11102-012-0420-x

[9] Johns, M.W. (1991) A New Method for Measuring Daytime Sleepiness: The Epworth Sleepiness Scale. *Sleep*, **14**, 540-545. https://doi.org/10.1093/sleep/14.6.540

[10] Epstein, L.J., Kristo, D., Strollo, P.J., Friedman, N., Malhotra, A., *et al.* (2009) Clinical Guideline for the Evaluation, Management and Long-Term Care of Obstructive Sleep Apnea in Adults. *Journal of Clinical Sleep Medicine*, **5**, 263-276.

[11] Commissione Paritetica Associazione Italiana Medicina del Sonno (AIMS), Associazione Italiana Pneumologi Ospedalieri (AIPO) (2001) Linee guida di procedura diagnostica nella sindrome delle apnee ostruttive nel sonno dell'adulto. http://www.sonnomed.it/linee_guida.htm

[12] Attal, P. and Chanson, P. (2010) Endocrine Aspects of Obstructive Sleep Apnea. *The Journal of Clinical Endocrinology & Metabolism*, **95**, E483-E495. https://doi.org/10.1210/jc.2009-1912

[13] Guo, X., Gao, L., Zhao, Y., Wang, M., Jiang, B., *et al.* (2018) Characteristics of the Upper Respiratory Tract with Acromegaly and Correlations with Obstructive Sleep Apnoea/Hypopnea Syndrome. *Sleep Medicine*, **48**, 27-34. https://doi.org/10.1016/j.sleep.2018.04.011

[14] De Menis, E., Giustina, A., Colao, A., Degli Uberti, E., Ghigo, E., *et al.* (2011) Assessment of the Awareness and Management of Sleep Apnea Syndrome in Acromegaly. The COM.E.TA (Comorbidities Evaluation and Treatment in Acromegaly) Italian Study Group. *Journal of Endocrinological Investigation*, **34**, 60-64. https://doi.org/10.1007/BF03346696

[15] Ceccato, F., Bernkopf, E. and Scaroni, C. (2015) Sleep Apnea Syndrome in Endocrine Clinics. *Journal of Endocrinological Investigation*, **38**, 827-834. https://doi.org/10.1007/s40618-015-0338-z

[16] Rosenow, F., McCarthy, V. and Caruso, A.C. (1998) Sleep Apnoea in Endocrine Diseases. *Journal of Sleep Research*, **7**, 3-11. https://doi.org/10.1046/j.1365-2869.1998.00086.x

[17] Cadieux, R.J., Kales, A., Santen, R.J., Bixler, E.O. and Gordon, R. (1982) Endoscopic Findings in Sleep Apnoea Associated with Acromegaly. *The Journal of Clinical Endocrinology & Metabolism*, **55**, 18-22. https://doi.org/10.1210/jcem-55-1-18

[18] Dostalova, S., Sonka, K., Smahel, Z., Weiss, V., Marek, J., *et al.* (2001) Craniofacial Abnormalities and Their Relevance for Sleep Apnoea Syndrome Aetiopathogenesis in Acromegaly. *European Journal of Endocrinology*, **144**, 491-497. https://doi.org/10.1530/eje.0.1440491

[19] Davi, M.V., Dalle Carbonare, L., Giustina, A., Ferrari, M., Frigo, A., *et al.* (2008) Sleep Apnoea Syndrome Is Highly Prevalent in Acromegaly and Only Partially Reversible after Biochemical Control of the Disease. *European Journal of Endocrinology*, **159**, 533-540. https://doi.org/10.1530/EJE-08-0442

[20] Roemmler, J., Gutt, B., Fischer, R., Vay, S., Wiesmeth, A., *et al.* (2012) Elevated Incidence of Sleep Apnoea in Acromegaly-Correlation to Disease Activity. *Sleep and Breathing*, **16**, 1247-1253. https://doi.org/10.1007/s11325-011-0641-7

[21] Weiss, V., Sonka, K., Pretl, M., Dosta'lova', S., Klozar, J., *et al.* (2000) Prevalence of the Sleep Apnea Syndrome in Acromegaly Population. *Journal of Endocrinological Investigation*, **23**, 515-519. https://doi.org/10.1007/BF03343767

[22] Tsoi, U.A., Sviryaev, Y.V., Korostovtseva, L.S., Semenov, A.P., Vaulina, D.A., *et al.* (2015) Clinical Features of Obstructive Sleep Apnea Syndrome in Patients with Acromegaly. *Terapevticheskii Arkhiv*, **87**, 47-52. https://doi.org/10.17116/terarkh201587447-52

[23] Brody, J.S., Fisher, A.B., Gocmen, A. and DuBois, A.B. (190) Acromegalic Pneumonomegaly: Lung Growth in the Adult. *Journal of Clinical Investigation*, **49**, 1051-1060. https://doi.org/10.1172/JCI106321

[24] Creutzberg, E.C. and Casaburi, R. (2003) Endocrinological Disturbances in Chronic Obstructive Pulmonary Disease. *European Respiratory Journal*, **46**, 76s-80s. https://doi.org/10.1183/09031936.03.00004610

[25] Vogiatzis, I., Stratakos, G., Simoes, D.C., Terzis, G., Georgiadou, O., *et al.* (2007) Effects of Rehabilitative Exercise on Peripheral Muscle TNF, IL-6, IGF-I and MyoD Expression in Patients with COPD. *Thorax*, **62**, 950-956. https://doi.org/10.1136/thx.2006.069310

[26] Glaser, J., Friedrich, N., Ewert, R., Schäper, C., Nauck, M., *et al.* (2009) Association between Serum Insulin-Like Growth Factor (IGF) I and IGF Binding Protein-3 and Lung Function. *The Journal of Clinical Endocrinology & Metabolism*, **94**, 2452-2458. https://doi.org/10.1210/jc.2008-2662

[27] Dobrucki, L.W., Tsutsumi, Y., Kalinowski, L., Dean, J., Gavin, M., *et al.* (2010) Analysis of Angiogenesis Induced by Local IGF-1 Expression after Myocardial Infarction Using MicroSPECT-CT Imaging. *Journal of Molecular and Cellular Cardiology*, **48**, 1071-1079. https://doi.org/10.1016/j.yjmcc.2009.10.008

[28] Rabinovsky, E.D. and Draghia-Akli, R. (2004) Insulin-Like Growth Factor I Plasmid Therapy Promotes *in Vivo* Angiogenesis. *Molecular Therapy*, **9**, 46-55. https://doi.org/10.1016/j.ymthe.2003.10.003

[29] Iandelli, I., Gorini, M., Duranti, R., Bassi, F., Misuri, G., *et al.* (1997) Respiratory Muscle Function and Control of Breathing in Patients with Acromegaly. *European Respiratory Journal*, **10**, 977-982. https://doi.org/10.1183/09031936.97.10050977

[30] Luboshitzky, R. and Barzilai, D. (1980) Hypoxemia and Pulmonary Function in Acromegaly. *The American Review of Respiratory Disease*, **121**, 471-475. https://doi.org/10.1164/arrd.1980.121.3.471

[31] Kamenicky, P., Viengchareun, S., Blanchard, A., Meduri, G., Zizzari, P., *et al.* (2008) Epithelial Sodium Channel Is a Key Mediator of Growth Hormone-Induced Sodium Retention in Acromegaly. *Endocrinology*, **149**, 3294-3305.

https://doi.org/10.1210/en.2008-0143

[32] Sicuro, A., Mahlbacher, K., Hulter, H.N. and Krapf, R. (1998) Effect of Growth Hormone on Renal and Systemic Acid-Base Homeostasis in Humans. *American Journal of Physiology*, **274**, F650-F657.
https://doi.org/10.1152/ajprenal.1998.274.4.F650

International Journal of Clinical Medicine, 2019, 10, 565-575
https://www.scirp.org/journal/ijcm
ISSN Online: 2158-2882
ISSN Print: 2158-284X

Exosomes in Sepsis Diagnosis and Treatment

Min Huang[1*], Huan Deng[1*], Jiang Li[2], Xingyu Tao[1], Baohui Jia[3#]

[1]The Third Affiliated Hospital of Nanchang University, Nanchang, China
[2]Zhengzhou Railway Vocational and Technical College/Henan Provincial Engineering Research Center of Natural Drug Extraction and Medical Technology Application, Zhengzhou, China
[3]The Fourth Affiliated Hospital of Nanchang University, Nanchang, China
Email: #bhjia@126.com

How to cite this paper: Huang, M., Deng, H., Li, J., Tao, X.Y. and Jia, B.H. (2019) Exosomes in Sepsis Diagnosis and Treatment. *International Journal of Clinical Medicine*, **10**, 565-575.
https://doi.org/10.4236/ijcm.2019.1010046

Received: September 9, 2019
Accepted: October 20, 2019
Published: October 23, 2019

Abstract

Sepsis has been redefined as a disorder of host response to infection, systemic circulation and cell/metabolic abnormalities. Exosomes are small (30 - 150 nm) vesicles produced by all cells under physiological and pathological conditions, with the potential to transfer proteins, lipids, small RNAs, messenger RNAs, or DNA between cells. Exosomes are natural cargoes for proteins, carbohydrates, nucleic acids and lipids. Exosomes play a central role in cellular communication and contribute to many pathophysiological processes, including immune responses and tumor progression. Exosomes have made great progress in many subject areas, and their potential role in sepsis is now being explored. In this review, several topics are mentioned. Firstly, we discuss the biological characteristics and functions of exosomes. Next, we focus on the diagnostic and therapeutic potential of exosomes in sepsis. Finally, we discuss some of the problems encountered by the current exosomes research institute. Therefore, the exosomes with combined diagnostic and therapeutic functions play a huge clinical application for the future research in sepsis.

Keywords

Exosomes, Sepsis, Biological Function, Targeted Vector, Inflammation

1. Introduction

Exosomes are natural carriers of many signaling molecules, including lipids, proteins, DNA, mRNA, miRNA, and siRNA. They can smoothly pass through the circulation without degration by enzymes and thus transmit important mediators between cells [1]. Multi-functional circulating exosomes with various subtypes have been identified in many lesions such as cardiovascular, infectious

*Co-first author.

and autoimmune diseases. In addition, exosomes are also involved in adaptive immune response through the regulation of antigen presentation [2]. Sepsis remains a global medical problem, early diagnosis of which can inhibit its progression and improve outcomes [3]. However, the initiation or suspension of clinical interventions is still suffering from the lack of markers with high sensitivity and specificity. Accumulating evidence suggests that exosomes can be served as an attractive candidate for the treatment of sepsis. This review summarizes the biological behavior and application of exosomes [4].

2. Formation and Composition of Exosomes

2.1. Formation of Exosomes

Exosomes are small vesicles covered by plasma membrane [5]. The formation process of exosomes is complex and orderly. The intravesicular membrane is firstly inwardly depressed to form luminal vesicles, then multivesicular bodies are formed. When these polyvesicles are fused with lysosomes, they are degraded or recirculated and fused to the membrane, and then induced by endosomal sorting complex required for transport (ESCRT). The internal buds form granular vesicles, which are released into the extracellular environment and called exosomes [6].

2.2. Composition of Exosomes

Exosomes are mainly composed of lipids, proteins, mRNA, miRNAs, and long non-coding RNAs (lncRNAs) [7]. These components are essential for the unique biological functions of exosomes. The hydrophilic and lipophilic outer shell of exosomesis valuable in shuttling through hydrophilic and hydrophobic structures in the body fluid circulation [8].Nucleic acids, including mRNA, microRNA, and lncRNA, can be absorbed by the recipient cells through fusion with the target cell membrane, thereby activating the signaling pathway and controlling the protein expression [9]. A variety of protein components on the surface of exosomes such as (CD9, CD63, CD81), Alix, HSP70, HSP90 and GTPase have a labeling effect.

2.3. Biological Roles in Exocytosis and Sepsis

The content function of the exosomes and the mechanical properties of the membrane are being unveiled [10]. The exosomal function of different subpopulations varies between different donor/recipient cells and the tissue microenvironment. Under the influence of pathophysiological factors, donor cells load specific cargoes such as functional RNAs (miRNAs and mRNAs) and proteins into exosomes. Exosomes transport these cargoes to the recipient cells, causing subsequent genetic and phenotype changes [11]. The intercellular communication mediated by exosomes in sepsis is mainly through activation of target cell surface receptors, which induces cells to cope with changes in the external environment. After fusion with the recipient cells, the contents of the vesicles, such

as microRNAs (miRNAs) or mRNAs and proteins, are transported to the cytoplasm [12]. **Table 1** identifies the current exosomes with diagnostic value for sepsis in the study of exosomes.

As long as the donor cells are free of apoptosis and necrosis in the body, the exosomes secreted by it can persist in body fluids and maintain a stable concentration.

As donor cells, whatever conditions, can secrete exosomes, the concentration of which remains steady. These unique features of exosomes make them ideal carriers for anti-sepsis drugs [13].

3. Exosomes and Sepsis

3.1. Exosomes Participate in the Onset Process of Sepsis

Sepsis is defined as a life-threatening organ dysfunction caused by the host's dysregulation of immune response. However, our current understanding of its process, including early diagnosis, treatment, and prognosis evaluation, is still

Table 1. Exosome in body fluids as biomarkers of sepsis.

Associated Protein, mRNAs or miR	Findings	Effects
miR-15a	Differentially expressed in adult a neonatal sepsis	Inhibits angiogenesis through direct targeting of VEGF and FGF [33]
miR-16a	in mice exposed to lipopolysaccharides	expression was shown to be up-regulated Following CLP [34]
miR-17	in the whole blood of mice after CLP	were significantly up-regulated in the microarray analysis in mice subcutaneously [35]
miR-20a/b	in the whole blood of mice after CLP	were significantly up-regulated in the microarray analysis in mice subcutaneously [35]
miR-21	in late sepsis of mice	with higher mortality in LPS-peritonitis model [36]
miR-146a	patients with severe sepsi	Predicted mortality and treatment outcomes induced by severe sepsis and sepsis.
miR-150	in septic patients	associated with survival rate in patients with sepsis [37]
miR-195	mice with CLP	only significantly expressed in the CLP model
miR-223	patients with mild sepsis severe sepsis and septic shock	as novel diagnostic biomarkers of sepsis [38]
ATF3	mice	an interesting sepsis-AKI biomarker [39]
SPTLC3	sepsis patient	potential classifier to monitor clinical progression of sepsis [40]

miR = microRNA, ATF3 = Activating transcription factor 3 RNA, SPTLC3 = Serine Palmitoyltransferase, Long Chain Base Subunit 3.

unclear. Almost all cells secrete exosomes, which are present throughout the body fluid circulation and mediate many aspects of disease development and progression by mediating cell-to-cell signaling [14] The currently evolving biotechnology of nanotechnology and genomics and proteomics provide the directions to target therapeutic agents in septic patients (**Table 2**).

In the setting of activation and apoptosis, exosomes are released and express specific membrane epitopes as their parental cells. The prospect for exosomes use in septic patients is bright, ranging from rapid and precise diagnostics [15]. Platelet-derived exosomes are isolated from patients with sepsis. These exosomes can produce oxidative enzymes in vascular endothelial cells and smooth muscle cells, induce apoptosis of vascular cells, suggesting a process of sepsis and septic shock. Platelet-derived exosomes may mediate vascular dysfunction through redox signaling pathways. During sepsis, platelets exposed to NO (nitric oxide) donors and LPS (lipopolysaccharide) secrete exosomes that activate caspase-3 and produce superoxide, NO and peroxynitrite. The base anion induces apoptosis, suggesting that platelet-derived exosomes can mediate vascular injury in a pathological, rather than physiological, environment. The anti-apoptotic effect

Table 2. Exosomes as therapeutic drug carriers and delivery vehicle.

Associated Protein, mRNAs or miR	Findings	Effects
miR-26b	mice with CLP	affected the antiviral response of the host
miR-27a	in the lungs of septic mice	up regulated in the progression to shock
miR-34a	in murine sepsis	Regulate vascular endothelial cell senescence
miR-106a/b	in whole blood of mice	Promote phagocytosis of macrophages by targeting SIRPa [41]
miR-126	in murine sepsis	Attenuates the increase in plasma levels of cytokines and chemokines induced by sepsis
NADPH oxidase	patients with diagnosis of septic shock	Improve dysfunction of heart and papillary muscles in patients with sepsis [42]
miR-223	in CLP mice	play an important role in MSC-induced cardioprotection in sepsis [43]
MFG-E8	rats by CLP	Reduce the level of inflammatory cytokines and increase the survival rate of experimental animals [44]
ADMSC	patients with diagnosis of septic shock	Improve dysfunction of heart and papillary muscles in patientswith sepsis [45]

miR = microRNA, NADPH oxidase = Nicotinamide-adenine dinucleotide-phosphate hydrogen oxidase, MFG-E8 = Milk-fat-globule epidermal-growth factor-factor VIII, ADMSC = Adipose-derived mesenchymal stem cell.

of plasma-derived circulating exosomes on T lymphocytes in patients with sepsis can down-regulate the mRNA and protein levels of pro-apoptotic genes，and up-regulate the mRNA and protein levels of anti-apoptotic genes. Finally, it inhibits apoptosis of septic cells. These findings suggest that tissue vascular injury, apoptosis, and inflammatory responses in sepsis/septic shock are closely related to exosomes released by various effector cells *in vivo* [16].

3.2. Exosomes Can Be Used as Biomarkers for the Diagnosis of Sepsis

The high mortality rate of sepsis is closely related to complications, and sepsis cardiomyopathy is the main complication. In sepsis, the vascular endothelial physiology changes due to damage to the cardiovascular system, such as decreased Ca^{2+} response, mitochondrial dysfunction, and decreased β-adrenergic receptor response. Exosomes play an important role in the pathophysiology of the disease. When platelets are activated in sepsis, exosomes containing high concentrations of NADPH are released into the blood vessels of the heart, leading to cardiac function damage and failure.

Exosomes are released from the cells into the blood circulation. By detecting the content of some exosomes-including proteins and RNA in the body fluids, some diseases can be predicted before onset of clinical symptoms [17]. There was a highly significant correlation between the transit of exosomes-derived miRNAs and inflammatory responses, oxidative stress, and cell cycle regulation in patients with septic shock [18]. Exosomes can be detected in the setting of trauma and stress and maintain a certain concentration level even in lethal illness. From organ dysfunction to failure, exosomes are likely to play a monitoring and regulatory role, and also play an important role in predicting the outcome of the disease. Exosomal expression can be detected in sepsis. When animals are exposed to Gram-negative or Gram-positive infections, miRNA-16, miRNA-17, miRNA-20a, miRNA-20b, miRNA-26a and miRNA-26b can be abnormally elevated [19]. The plasma expression levels of miR-15a, miR-27a and miR-34a in exosomes are also closely related to the severity of sepsis development. These expressions of miRNA can be consistent with the changes of patients with sepsis. The level of miRNA in the plasma of patients with severe sepsis complicated with shock may change, which may provide more value for further study of the pathogenesis of sepsis endothelial dysfunction.

To this end, if the expression of miRNA is quantified, foreign miRNA expression can be used as a good biomarker for evaluating, monitoring and optimizing intensive treatment of sepsis in future critically ill patients.

3.3. Exosomes Are Expected to Be a New Treatment for Sepsis

Exosomes have the biological activity of substance transfer between cells, with the great potential of becoming therapeutic drug carriers. Exosomes can act as a mediator between cells and organs, regulating the biological activities of various substances involved in communication and signaling between cells. By upregu-

lating miR-21 to increase its expression, it can reduce apoptosis and reduce the production of pro-inflammatory factors. Protecting organ damage in local and distal areas of sepsis may be a potential treatment for sepsis [20]. As an important inclusion in exosomes, miRNA is involved in the regulation of each cellular process. Their expression changes are related to the occurrence, development and repair of many diseases and are important regulators in physiological and pathological processes. MiR-21 may act as a protective molecular medium between damaged tissues and organs. It is expected to be a targeted treatment for sepsis.

MiR-146a enhances the effect of IL-1β on macrophage anti-sepsis infection. MiR-146a is a relatively important anti-inflammatory miRNA, and this exosome MiR-146a is greatly up-regulated due to inflammatory irritation.MiR-146a improves sexual performance and improves survival in mice with sepsis [21]. Although the mechanism for this study has not yet been fully elucidated, it can provide a new treatment for inflammatory disorders.MiR-145 can ameliorate sepsis-induced lung injury by inhibiting TGFBR2 signaling, and attenuate LPS-induced inflammation in mice by down-regulating IL-2 and TNF-α secretion, The overall survival rate of sepsis mice with lung injury was prolonged [22].

The circulating exosomes are rich in various types of biomolecules, affecting myocardial cell function, reducing the degree of myocardial infarction, reducing myocardial ischemia-reperfusion injury, and promoting myocardial regeneration and repair. And it is expected to be a specific biomarker for cardiovascular disease diagnosis, risk stratification and prognosis [23].

The use of proteins and nucleic acid components in exosomes as drug carriers and targeting tools for the treatment of sepsis is still being studied and discovered. The above studies suggest that it is effective to increase the content of some protective exosomes, thus avoiding the occurrence of multiple organ failure in sepsis.

3.4. Exosomes Play a Role in Important Organ Damage in Patients with Sepsis

Recently, sepsis has been redefined as a disorder of host response to infection, systemic circulation and cell/metabolic abnormalities, and its severity and clinical treatment depend largely on the organ dysfunction state of the patient. Every time an organ failure occurs, the probability of death will increase significantly [24]. Exosomes are released at different concentrations in healthy subjects and diseased populations, and the concentrations released into the circulation in different diseases are also different. Intratracheal instillation of extracellular vesicles derived from bone marrow mesenchymal stem cells can alleviate lung inflammation and edema caused by acute lung injury by inducing expression of keratinocyte growth factor [25]. The plasma concentrations of N-terminal B-type natriuretic peptide (NT-proBNP) and hypersensitive troponin T (hs-cTnT) in circulating blood in patients with severe sepsis or septic shock can follow the occur-

rence of acute myocardial infarction, therefore, exosomes can be used as a multifunctional clinical indicator of early ischemic myocardial injury in intensive care [26].

Zhou found in the experiment that exosomes miR-126-5p and 3p can inhibit LPS-induced human microvascular endothelial cells (HMVECs) high mobility group protein b1 (HMGB1) and vascular cell adhesion molecule 1 (VCAM1) levels, It was confirmed that the use of EPR exosomes to deliver miR-126 can prevent microvascular dysfunction, attenuate the increase in plasma cytokine and chemokine levels induced by sepsis, inhibit lung and renal vascular leakage in vital organs, and improve the survival rate of mice with sepsis [27]. In the mouse kidney ischemia/reperfusion injury model, the level of urinary extracellular ATF3 (activating transcription factor 3) was detected to be higher than normal before serum creatinine concentration had increased [28]. This finding suggests that extracellular ATF3 is more sensitive as a biomarker in early acute kidney injury than traditional clinical biomarkers, suggesting that exosomes are expected to be an effective tool for the diagnosis/treatment of early acute kidney injury in further studies.

4. Current Problems

However, there are many problems and challenges in the study of exosomes. How to separate pure exosomes from various body fluids is a key issue. Presently, exosome extraction methods are mainly in five categories, namely, ultracentrifugation, precipitation, immunoadsorption, ultrafiltration, and microfluidic separation techniques [29]. Although differential centrifugation and ultracentrifugation are the most commonly used techniques in clinical laboratories. However, this method has certain limitations due to its protein contamination and yield problems [30]. New methods such as density gradient centrifugation, while overcoming the above limitations, are complicated in steps, and the experimental process takes a long time and cannot be applied to the clinic. There are many reports on the relationship between extracellular vesicle biomarkers and various diseases. However, the results of individual studies vary widely, probably due to the differences of exosome in extraction and purification methods [31]. Due to the complex structure, variable composition and versatility of exosomes, exosomes in these natural states are difficult to be used as targeting targets for drugs. At the same time, most of these studies are preliminary, only in animal models, and further research is needed to translate into clinical applications.

5. Conclusion and Outlook

Although the pathogenesis and clinical treatment of sepsis have made great progress, the mortality rate of sepsis has not decreased [32]. So far, some indicators of sepsis have been used only to determine the presence of organ failure and to assess the patient's clinical outcome. Exosomes can play a huge potential as a disease-specific biomarker and a carrier for the treatment of sepsis/septic shock.

It is a hot research topic to make full use of the unique biological function of exosomes and to study and elucidate the mechanism of sepsis from different levels such as cells and molecules. It will provide new help for targeted specific treatment of sepsis/infectious shock.

Acknowledgements

This work is supported by National Natural Science Foundation of China (81760353), Science and Technology Project of Henan Province (No. 172102 310627), Major Science research Project of high Education of Henan Province (No. 16A320065, 20A320086), Major Science research Project of Zhengzhou Railway Vocational and Technical College (No. 2019KY002).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Yanez-Mo, M., Siljander, P.R., Andreu, Z., *et al.* (2015) Biological Properties of Extracellular Vesicles and Their Physiological Functions. *Journal of Extracellular Vesicles*, **4**, 27065-27066.

[2] De Toro, J., Herschlik, L., Waldner, C., *et al.* (2015) Emerging Roles of Exosomes in Normal and Pathological Conditions: New Insights for Diagnosis and Therapeutic Applications. *Frontiers in Immunology*, **6**, 203. https://doi.org/10.3389/fimmu.2015.00203

[3] Manu, S., Gary, S.P., Mitchell, L.L., *et al.* (2016) Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*, **315**, 762-774. https://doi.org/10.1001/jama.2016.0289

[4] Terrasini, N. and Lionetti, V. (2017) Exosomes in Critical Illness. *Critical Care Medicine*, **45**, 1054-1060. https://doi.org/10.1097/CCM.0000000000002328

[5] Johnstone, R.M., Adam, M., Hammond, J.R., *et al.* (1987) Vesicle Formation during Reticulocyte Maturation—Association of Plasma-Membrane Activities with Released Vesicles (Exosomes). *Journal of Biological Chemistry*, **262**, 9412-9420.

[6] Buschow, S.I., Nolte-'T Hoen, E.N.M., van Niel, G., *et al.* (2009) MHC II in Dendritic Cells Is Targeted to Lysosomes or T Cell-Induced Exosomes via Distinct Multivesicular Body Pathways. *Traffic*, **10**, 1528-1542. https://doi.org/10.1111/j.1600-0854.2009.00963.x

[7] Shahabipour, F., Barati, N., Johnston, T.P., *et al.* (2017) Exosomes: Nanoparticulate Tools for RNA Interference and Drug Delivery. *Journal of Cellular Physiology*, **232**, 1660-1668. https://doi.org/10.1002/jcp.25766

[8] Ren, J., He, W., Zheng, L., *et al.* (2016) From Structures to Functions: Insights into Exosomes as Promising Drug Delivery Vehicles. *Biomaterials Science*, **4**, 910-921. https://doi.org/10.1039/C5BM00583C

[9] Valadi, H., Ekstrom, K., Bossios, A., *et al.* (2007) Exosome-Mediated Transfer of mRNAs and microRNAs Is a Novel Mechanism of Genetic Exchange between Cells. *Nature Cell Biology*, **9**, 654-672. https://doi.org/10.1038/ncb1596

[10] Lucia, P., Andrea, Z. and Annalisa, R. (2018) Biophysical Properties of Extracellular Vesicles in Diagnostics. *Biomarkers in Medicine*, **12**, 383-391. https://doi.org/10.2217/bmm-2017-0458

[11] Wu, J., Wang, Y. and Li, L. (2017) Functional Significance of Exosomes Applied in Sepsis: A Novel Approach to Therapy. *Biochimica et Biophysica Acta—Molecular Basis of Disease*, **1863**, 292-297. https://doi.org/10.1016/j.bbadis.2016.10.024

[12] Srinivasan, S., Vannberg, F.O. and Dixon, J.B. (2016) Lymphatic Transport of Exosomes as a Rapid Route of Information Dissemination to the Lymph Node. *Scientific Reports*, **6**, Article No. 24436. https://doi.org/10.1038/srep24436

[13] Conlan, R.S., Pisano, S., Oliveira, M.I., *et al*. (2017) Exosomes as Reconfigurable Therapeutic Systems. *Trends in Molecular Medicine*, **23**, 636-650. https://doi.org/10.1016/j.molmed.2017.05.003

[14] Takahashi, A., Okada, R., Nagao, K., *et al*. (2017) Exosomes Maintain Cellular Homeostasis by Excreting Harmful DNA from Cells. *Nature Communications*, **8**, 15287. https://doi.org/10.1038/ncomms15287

[15] Pierre, R., Johannes, Z. and Susanne, D. (2018) Extracellular Vesicles as Markers and Mediators in Sepsis. *Theranostics*, **8**, 3348-3365. https://doi.org/10.7150/thno.23453

[16] Gambim, M.H., Do Carmo, A.D.O., Marti, L., *et al*. (2007) Platelet-Derived Exosomes Induce Endothelial Cell Apoptosis through Peroxynitrite Generation: Experimental Evidence for a Novel Mechanism of Septic Vascular Dysfunction. *Critical Care*, **11**, R107. https://doi.org/10.1186/cc6133

[17] Revenfeld, A.L.S., Baek, R., Nielsen, M.H., *et al*. (2014) Diagnostic and Prognostic Potential of Extracellular Vesicles in Peripheral Blood. *Clinical Therapeutics*, **36**, 830-846. https://doi.org/10.1016/j.clinthera.2014.05.008

[18] Juliana, M.R., Ludmila, R.P.F., Gustavo, H.E., *et al*. (2018) Exosomes from Patients with Septic Shock Convey miRNAs Related to Inflammation and Cell Cycle Regulation: New Signaling Pathways in Sepsis? *Critical Care* (*London*, *England*), **22**, 68. https://doi.org/10.1186/s13054-018-2003-3

[19] Wu, S., Yang, J.C., Rau, C., *et al*. (2013) Profiling Circulating microRNA Expression in Experimental Sepsis Using Cecal Ligation and Puncture. *PLoS ONE*, **8**, e77936. https://doi.org/10.1371/journal.pone.0077936

[20] Jia, P., Wu, X., Dai, Y., *et al*. (2017) MicroRNA-21 Is Required for Local and Remote Ischemic Preconditioning in Multiple Organ Protection against Sepsis. *Critical Care Medicine*, **45**, E703-E710. https://doi.org/10.1097/CCM.0000000000002363

[21] Song, Y., Dou, H., Li, X., *et al*. (2017) Exosomal miR-146a Contributes to the Enhanced Therapeutic Efficacy of Interleukin-1-Primed Mesenchymal Stem Cells against Sepsis. *Stem Cells*, **35**, 1208-1221. https://doi.org/10.1002/stem.2564

[22] Cao, X.H., *et al*. (2019) MiR-145 Negatively Regulates TGFBR2 Signaling Responsible for Sepsis-Induced Acute Lung Injury. *Biomedicine & Pharmacotherapy*, **111**, 852-858. https://doi.org/10.1016/j.biopha.2018.12.138

[23] Ebru, K., Steffen, U.E., Julia, H., *et al*. (2018) Extracellular Vesicles: Packages Sent with Complement. *Frontiers in Immunology*, **9**, 721. https://doi.org/10.3389/fimmu.2018.00721

[24] Schmoch, T., *et al*. (2017) New Sepsis-3 Definition: Do We Have to Treat Sepsis before We Can Diagnose It from Now on? *Der Anaesthesist*, **66**, 614-621.

[25] Zhu, Y., Feng, X., Abbott, J., *et al*. (2014) Human Mesenchymal Stem Cell Microvesicles for Treatment of *Escherichia coli* Endotoxin-Induced Acute Lung Injury in

Mice. *Stem Cells*, **32**, 116-125. https://doi.org/10.1002/stem.1504

[26] Masson, S., Caironi, P., Fanizza, C., *et al*. (2016) Sequential N-Terminal Pro-B-Type Natriuretic Peptide and High-Sensitivity Cardiac Troponin Measurements during Albumin Replacement in Patients with Severe Sepsis or Septic Shock. *Critical Care Medicine*, **44**, 707-716.

[27] Zhou, Y., *et al*. (2018) Exosomes from Endothelial Progenitor Cells Improve the Outcome of a Murine Model of Sepsis. *Molecular Therapy*: *The Journal of the American Society of Gene Therapy*, **26**, 1375-1384. https://doi.org/10.1016/j.ymthe.2018.02.020

[28] Chen, H., Lai, P., Lan, Y., *et al*. (2014) Exosomal ATF3 RNA Attenuates Pro-Inflammatory Gene MCP-1 Transcription in Renal Ischemia-Reperfusion. *Journal of Cellular Physiology*, **229**, 1202-1211. https://doi.org/10.1002/jcp.24554

[29] Hyungsoon, I., Cesar, M.C., *et al*. (2018) New Technologies for Analysis of Extracellular Vesicles. *Chemical Reviews*, **118**, 1917-1950. https://doi.org/10.1021/acs.chemrev.7b00534

[30] Lotvall, J., Hill, A.F., Hochberg, F., *et al*. (2014) Minimal Experimental Requirements for Definition of Extracellular Vesicles and Their Functions: A Position Statement from the International Society for Extracellular Vesicles. *Journal of Extracellular Vesicles*, **3**, 26913. https://doi.org/10.3402/jev.v3.26913

[31] Barile, L. and Vassalli, G. (2017) Exosomes: Therapy Delivery Tools and Biomarkers of Diseases. *Pharmacology & Therapeutics*, **174**, 63-78. https://doi.org/10.1016/j.pharmthera.2017.02.020

[32] Angus, D.C. and van der Poll, T. (2013) Severe Sepsis and Septic Shock. *The New England Journal of Medicine*, **369**, 2063. https://doi.org/10.1056/NEJMc1312359

[33] Wang, X.L., *et al*. (2015) miR-15a/16 Are Upreuglated in the Serum of Neonatal Sepsis Patients and Inhibit the LPS-Induced Inflammatory Pathway. *International Journal of Clinical and Experimental Medicine*, **8**, 5683-5690.

[34] Hsieh, C.-H., *et al*. (2012) Whole Blood-Derived microRNA Signatures in Mice Exposed to Lipopolysaccharides. *Journal of Biomedical Science*, **19**, Article No. 69. https://doi.org/10.1186/1423-0127-19-69

[35] Wu, S.C., Yang, J.C., Rau, C.S., Chen, Y.C., Lu, T.H., *et al*. (2013) Profiling Circulating microRNA Expression in Experimental Sepsis Using Cecal Ligation and Puncture. *PLoS ONE*, **8**, e77936. https://doi.org/10.1371/journal.pone.0077936

[36] Rebecca, E.B., Daniel, J.C., Lindsey, R., *et al*. (2016) Anti-Inflammatory Effects of miR-21 in the Macrophage Response to Peritonitis. *Journal of Leukocyte Biology*, **99**, 361-371. https://doi.org/10.1189/jlb.4A1014-489R

[37] Christoph, R., Mark, L., David, V.C., *et al*. (2013) Circulating microRNA-150 Serum Levels Predict Survival in Patients with Critical Illness and Sepsis. *PLoS ONE*, **8**, e54612. https://doi.org/10.1371/journal.pone.0054612

[38] Wang, H.J., Zhang, P.J., Chen, W.J., Feng, D., Jia, Y.H. and Xie, L.X. (2012) Four Serum microRNAs Identified as Diagnostic Biomarkers of Sepsis. *The Journal of Trauma and Acute Care Surgery*, **73**, 850-854. https://doi.org/10.1097/TA.0b013e31825a7560

[39] Tanaporn, P., Wiwat, C., Poorichaya, S., *et al*. (2017) Urinary Exosomal Activating Transcriptional Factor 3 as the Early Diagnostic Biomarker for Sepsis-Induced Acute Kidney Injury. *BMC Nephrology*, **18**, 10. https://doi.org/10.1186/s12882-016-0415-3

[40] Xu, Y., Ku, X., Wu, C., Cai, C., Tang, J. and Yan, W. (2018) Exosomal Proteome

Analysis of Human Plasma to Monitor Sepsis Progression. *Biochemical and Biophysical Research Communications*, **499**, 856-861. https://doi.org/10.1016/j.bbrc.2018.04.006

[41] Zhu, D., Pan, C., Li, L., *et al*. (2013) MicroRNA-17/20a/106a Modulate Macrophage Inflammatory Responses through Targeting Signal-Regulatory Protein *a*. *The Journal of Allergy and Clinical Immunology*, **132**, 426-436.e8. https://doi.org/10.1016/j.jaci.2013.02.005

[42] Luciano, C.P.A., Mariano, J., Vera, P., *et al*. (2007) Platelet-Derived Exosomes from Septic Shock Patients Induce Myocardial Dysfunction. *Critical Care* (*London*, *England*), **11**, R120. https://doi.org/10.1186/cc6176

[43] Wang, X., Gu, H., Qin, D., *et al*. (2015) Exosomal miR-223 Contributes to Mesenchymal Stem Cell-Elicited Cardioprotection in Polymicrobial Sepsis. *Scientific Reports*, **5**, Article No. 13721. https://doi.org/10.1038/srep13721

[44] Miksa, M., Wu, R., Dong, W., Das, P., Yang, D. and Wang, P. (2006) Dendritic Cell-Derived Exosomes Containing Milk Fat Globule Epidermal Growth Factor-Factor VIII Attenuate Proinflammatory Responses in Sepsis. *Shock*, **25**, 586-593. https://doi.org/10.1097/01.shk.0000209533.22941.d0

[45] Chang, C.L., Sung, P.H., Chen, K.H., *et al*. (2018) Adipose-Derived Mesenchymal Stem Cell-Derived Exosomes Alleviate Overwhelming Systemic Inflammatory Reaction and Organ Damage and Improve Outcome in Rat Sepsis Syndrome. *American Journal of Translational Research*, **10**, 1053-1070.

Call for Papers

International Journal of Clinical Medicine

ISSN: 2158-284X (Print) ISSN: 2158-2882 (Online)
https://www.scirp.org/journal/ijcm

International Journal of Clinical Medicine (IJCM) is a peer reviewed journal dedicated to the latest advancement of clinical medicine. The goal of this journal is to keep a record of the state-of-the-art research and to promote study, research and improvement within its various specialties.

Subject Coverage

The journal publishes original papers including but not limited to the following fields:

- Allergy and Clinical Immunology
- Cancer Research and Clinical Oncology
- Clinical Anaesthesiology
- Clinical Anatomy
- Clinical and Applied Thrombosis/Hemostasis
- Clinical and Experimental Allergy
- Clinical and Experimental Dermatology
- Clinical and Experimental Hypertension
- Clinical and Experimental Immunology
- Clinical and Experimental Medicine
- Clinical and Experimental Metastasis
- Clinical and Experimental Nephrology
- Clinical and Experimental Ophthalmology
- Clinical and Experimental Optometry
- Clinical and Experimental Otorhinolaryngology
- Clinical and Experimental Pathology
- Clinical and Experimental Pharmacology and Physiology
- Clinical and Molecular Allergy
- Clinical and Translational Oncology
- Clinical Anesthesia
- Clinical Apheresis
- Clinical Autonomic Research
- Clinical Biochemistry and Nutrition
- Clinical Biomechanics
- Clinical Cardiology
- Clinical Case Studies
- Clinical Child Psychology and Psychiatry
- Clinical Chiropractic
- Clinical Densitometry
- Clinical Effectiveness in Nursing
- Clinical Endocrinology and Metabolism
- Clinical Epidemiology
- Clinical Forensic Medicine
- Clinical Gastroenterology and Hepatology
- Clinical Genetics
- Clinical Haematology
- Clinical Hypertension
- Clinical Imaging
- Clinical Immunology
- Clinical Implant Dentistry and Related Research
- Clinical Interventions in Aging
- Clinical Laboratory Analysis
- Clinical Linguistics & Phonetics
- Clinical Lipidology
- Clinical Microbiology and Antimicrobials
- Clinical Microbiology and Infection
- Clinical Microbiology and Infectious Diseases
- Clinical Molecular Pathology
- Clinical Monitoring and Computing
- Clinical Neurology and Neurosurgery
- Clinical Neurophysiology
- Clinical Neuropsychology
- Clinical Neuroradiology
- Clinical Neuroscience
- Clinical Nursing
- Clinical Nutrition
- Clinical Obstetrics and Gynaecology
- Clinical Oncology and Cancer Research
- Clinical Ophthalmology
- Clinical Oral Implants Research
- Clinical Oral Investigations
- Clinical Orthopaedics and Related Research
- Clinical Otolaryngology
- Clinical Pathology
- Clinical Pediatric Emergency Medicine
- Clinical Periodontology
- Clinical Pharmacology & Toxicology
- Clinical Pharmacy and Therapeutics
- Clinical Physiology and Functional Imaging
- Clinical Practice and Epidemiology in Mental Health
- Clinical Psychology and Psychotherapy
- Clinical Psychology in Medical Settings
- Clinical Radiology
- Clinical Rehabilitation
- Clinical Research and Regulatory Affairs
- Clinical Research in Cardiology
- Clinical Respiratory
- Clinical Rheumatology
- Clinical Simulation in Nursing
- Clinical Sleep Medicine
- Clinical Techniques in Small Animal Practice
- Clinical Therapeutics
- Clinical Toxicology
- Clinical Transplantation
- Clinical Trials
- Clinical Ultrasound
- Clinical Virology
- Complementary Therapies in Clinical Practice
- Consulting and Clinical Psychology
- Contemporary Clinical Trials
- Controlled Clinical Trials
- Diabetes Research and Clinical Practice
- Evaluation in Clinical Practice
- Fundamental & Clinical Pharmacology
- Hereditary Cancer in Clinical Practice
- Human Psychopharmacology: Clinical and Experimental
- Innovations in Clinical Neuroscience
- Laboratory and Clinical Medicine
- Neurophysiologie Clinique/Clinical Neurophysiology
- Nutrition in Clinical Practice
- Pacing and Clinical Electrophysiology
- Psychiatry in Clinical Practice
- Therapeutics and Clinical Risk Management
- Veterinary Clinical Pathology

We are also interested in short papers (letters) that clearly address a specific problem, and short survey or position papers that sketch the results or problems on a specific topic. Authors of selected short papers would be invited to write a regular paper on the same topic for future issues of the ***IJCM***.

Notes for Intending Authors

All manuscripts submitted to IJCM must be previously unpublished and may not be considered for publication elsewhere at any time during IJCM's review period. Paper submission will be handled electronically through the website. All papers are refereed through a peer review process. Additionally, accepted ones will immediately appear online followed by printed in hard copy. For more details about the submissions, please access the website.

Website and E-Mail

https://www.scirp.org/journal/ijcm Email: ijcm@scirp.org

What is SCIRP?

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

What is Open Access?

All original research papers published by SCIRP are made freely and permanently accessible online immediately upon publication. To be able to provide open access journals, SCIRP defrays operation costs from authors and subscription charges only for its printed version. Open access publishing allows an immediate, worldwide, barrier-free, open access to the full text of research papers, which is in the best interests of the scientific community.

- High visibility for maximum global exposure with open access publishing model
- Rigorous peer review of research papers
- Prompt faster publication with less cost
- Guaranteed targeted, multidisciplinary audience

Scientific Research Publishing

Website: https://www.scirp.org

Subscription: sub@scirp.org

Advertisement: service@scirp.org