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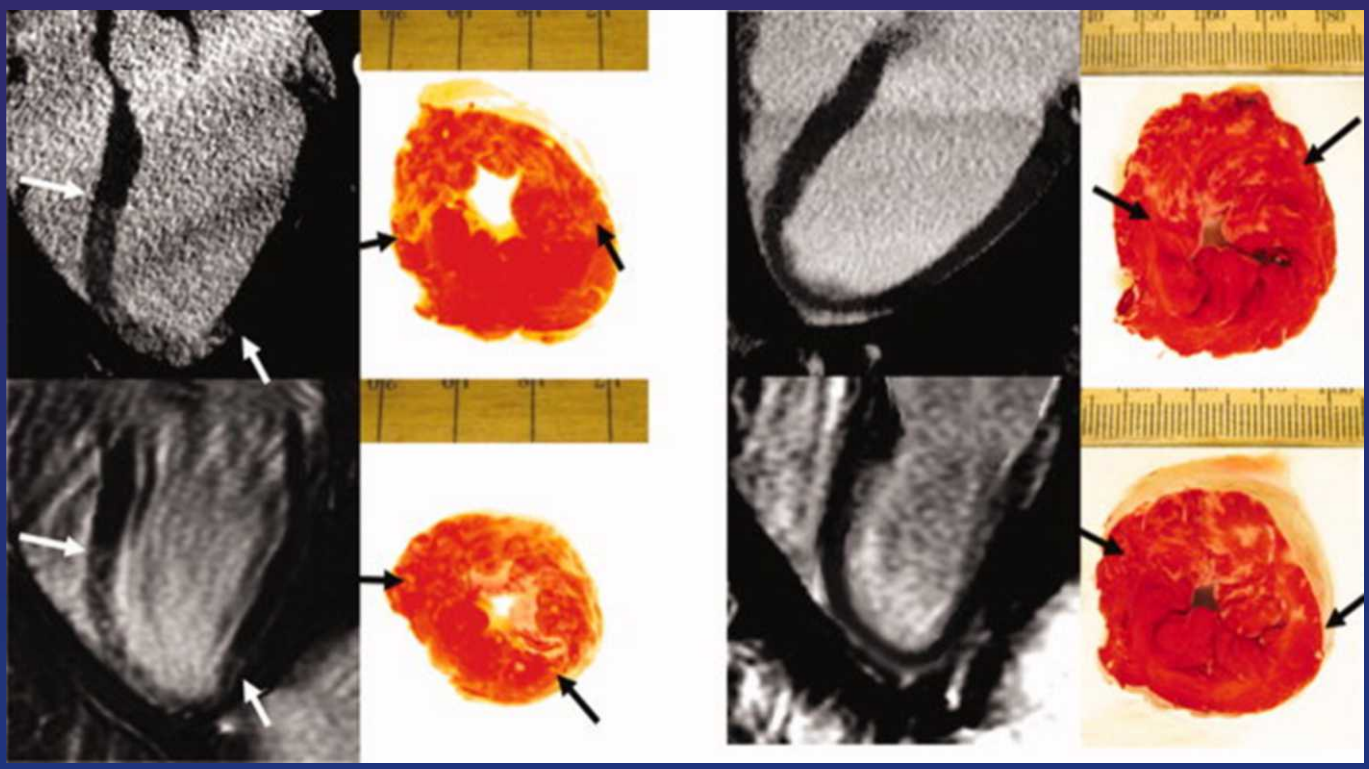

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The Effects of Dexmedetomidine Continuous Rate Infusion (CRI) on Isoflurane Anaesthesia in Healthy Horses

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Abstract

The concept of the modern anaesthesiological technique partial intravenous anaesthesia (PIVA) is by means of infusion of different pharmacological agents as a supplement to inhalation anaesthetics to reduce the concentration of volatile agents maintaining surgical anaesthesia and to decrease their noxious side effects mainly on cardiovascular and respiratory systems. Alpha-2 agonists are agents with frequent use in equine practice either as sedatives or in general anaesthesia PIVA protocols. The most selective amongst them, dexmedetomidine, is characterized by fewer side effects, lower doses, and fast elimination which make it appropriate for application as a continuous rate infusion (CRI). The purpose of this study was to trace out the effects of dexmedetomidine continuous rate infusion (CRI) as part of PIVA using isoflurane on volatile agent requirements, cardiovascular function, respiration and coagulation parameters, and recovery in healthy horses. Six healthy horses with average age 9.0 ± 5.1 year and mean body weight 247.7 ± 71.4 kg were subjected to either 3-hour lasting isoflurane or isoflurane-dexmedetomidine anaesthesia two weeks apart. The main clinical and anaesthesiological parameters were monitored in 10 minutes intervals. Electrolytes, acid-base, blood gases, and coagulation parameters were measured at the beginning and at the end of each anaesthesia. Recovery times and qualities were also recorded. The results showed that, the addition of dexmedetomidine by CRI at 1.75 μg·kg^{-1}·hour^{-1} to isoflurane anaesthesia slightly reduced isoflurane requirement for maintenance of surgical anaesthesia but had negligible effects on the recovery time and quality. PIVA using dexmedetomidine and isoflurane produced respiratory acidosis similar to isoflurane anaesthesia alone but with significantly more pronounced hypoxaemia and hyperlactaemia. Both investigated anaesthesia protocols did not influence significantly haemocoagulation parameters.

Keywords

Isoflurane, Dexmedetomidine, Continuous Rate Infusion, Horses

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1. Introduction

Anaesthesia related mortality in horses is much higher than other species [1] [2] which makes anaesthetists to look after safer protocols for general anaesthesia. Equid size, behavior and physiology contribute to significant risks and challenges to inhalation anaesthesia in horses relative to other species.

Inhalation agents provide deep and long enough anaesthesia for surgical procedures but on the price of pronounced cardiorespiratory depression [3]. Isoflurane is the most widely used volatile agent in equine anaesthesia because of its potency, low blood solubility, and low price. As other inhalation anaesthetics, isoflurane causes dose- and time-dependent cardiovascular and respiratory depression. Infusion of sedative, analgesic, or anaesthetic drugs in addition to inhalation agent can provide a surgical plane of anaesthesia using reduced doses and therefore may decrease undesired side effects. This technique in called partial intravenous anaesthesia (PIVA) and several agents has been studied and proposed to be used by continuous rate infusion (CRI) to supplement volatile agents in horses [4] [5].

Alpha 2-adrenoceptor agonists are widely used in equine anaesthesia including as a part of PIVA. They are potent sedatives with good analgesic properties and also have side effects after bolus administration such as bradycardia, arrhythmias, decreases in cardiac output, and increases in systemic vascular resistance, respiratory depression, decreasing intestinal motility, and ataxia. The use of IV alpha-2 agonists in balanced anaesthetic techniques has become more and more popular, mainly to reduce MAC of volatile agents and to improve recovery during the perioperative period without significant cardiorespiratory effects.

Xylazine, the least selective alpha-2 agonist (selectivity ratio $\alpha 2{:}\alpha 1 = 160{:}1$) has been shown to reduce MAC of isoflurane by 25% and 34% following IV administration of 0.5 and 1 $mg{\cdot}kg^{-1}$ respectively [6]. Pöpel *et al*. [7] reported that xylazine applied as a CRI at rate 1 $mg{\cdot}kg^{-1}{\cdot}hour^{-1}$ after bolus of 0.6 $mg{\cdot}kg^{-1}$ in isoflurane anaesthetized horses led to drop in anesthetic requirements and less need for blood pressure support compared to isoflurane alone.

A CRI of detomidine ($\alpha 2{:}\alpha 1 = 260{:}1$) at 5 $\mu g{\cdot}kg^{-1}{\cdot}hour^{-1}$ after bolus of 10 $\mu g{\cdot}kg^{-1}$ administered in isoflurane anesthetized horses undergoing to elective surgeries demonstrated typical cardiovascular effects without any beneficial influence on isoflurane requirements, recovery duration and quality [8].

The use of romifidine ($\alpha 2{:}\alpha 1 = 340{:}1$) as a CRI in isoflurane anesthetized horses submitted to elective surgeries was also described with contradictory results. According to Kuhn *et al*. [9] a CRI of 18 $\mu g{\cdot}kg^{-1}{\cdot}hour^{-1}$ after initial dose of 80 $\mu g{\cdot}kg^{-1}$ caused a significant reduction in concentration of inhaled agent with improved cardiovascural and respiratory parameters. In contrast, Devisscher *et al*. [10] failed to detect neither inhalation sparing effect nor better cardiopulmonary function and recovery quality, when using the same loading dose of romifidine followed by a CRI at 40 $\mu g{\cdot}kg^{-1}{\cdot}hour^{-1}$.

Medetomidine is an extremely selective alpha-2 agonist ($\alpha 2{:}\alpha 1 = 1620{:}1$) and lower side effects could be assumed. PIVA using isoflurane and CRI of medetomidine has been studied widely in the equine anaesthesia. The application of 7 $\mu g{\cdot}kg^{-1}$ IV followed by a CRI at 3.5 $\mu g{\cdot}kg^{-1}{\cdot}hour^{-1}$ in addition to isoflurane anaesthesia during orthopaedicsurgeries was found to reduce EtIso by 20% with lower dobutamine requirements to maintain arterial blood pressure and no differences in respiratory parameters comparatively to volatile anaesthetic alone [11].

Dexmedetomidine, the active enantiomer of medetomidine, is the most selective alpha-2 agonist ($\alpha 2{:}\alpha 1 = 3240{:}1$). It possesses beneficial pharmacokinetic properties such as short half-life and rapid distribution which render its use as a CRI very appropriate [12]. Administration of dexmedetomidine as an adjunctive agent to isoflurane anaesthesia produced an anaesthetic—sparing effects in human [13] [14] and dogs [15]. Its effects were studied on isoflurane anaesthetized horses by Marcilla *et al*. [16] [17]. In the former investigation the authors found out that the two CRIs used (1 and 1.75 $\mu g{\cdot}kg^{-1}{\cdot}hour^{-1}$) produced similar, small cardiopulmonary effects within an acceptable clinical range; in the former study they claimed that dexmedetomidine CRI at 1.75 $\mu g{\cdot}kg^{-1}{\cdot}hour^{-1}$ after a bolus of 3.5 $\mu g{\cdot}kg^{-1}$ did not reduce EtIso but improved recovery quality.

In the present study, we tested the effects of dexmedetomidine CRI on isoflurane requirements for maintenance of surgical anaesthesia as well as its influence on cardiopulmonary function, haemocoagulation and recovery in clinically healthy horses.

2. Material and Methods

2.1. Animals

The study was approved by the Ethical Committee of the Faculty of Veterinary Medicine at Trakia University in

Stara Zagora.

Six healthy horses from local primitive Karakachan breed with average age 9.0 ± 5.1 year and mean (± SD) body weight 247.7 ± 71.4 kg were included in the trial. Animals were bought from owners who intended to send them to slaughter house. They were kept in identical living and feeding conditions for a month before starting the experiment in order to eliminate the effect of environmental stress on the invest had variables. During this period a routine anthelmintic treatment was given using fenbendazole (Panacur®, Intervet) at a dose of 7.5 mg·kg^{-1} PO or mebendazol (Telmin®, Janssen Pharmaceutical, Belgium) at the same dose. Clinical and laboratory investigations were also preliminarily performed and all animals were allocated in ASA grade I or II thereafter. Horses were assigned first to control/saline (ISO) group and next (two weeks apart) to experimental/dexmedetomidine (ISOD) group in order to produce cross-over design. Food but not water was deprived 12 hours before general anaesthesia.

2.2. Experimental Design

Experimental design was similar to our previously accomplished experiment using PIVA with halothane and dexmedetomidine CRI in horses [18].

Acepromazine maleate (Neurotranq®, Alfasan International, Holland) was given IV at dose 0.03 mg·kg^{-1} as a premedication agent in the two groups. Xylazine hydrochloride (Alfasan International, Holland) 0.8 mg·kg^{-1} was administered IV thirty minutes later through one of the two 14—gauge 2.1 × 50 mm catheters (Venocan plus®, Kruuse, Denmark) placed in both jugular veins.

Anaesthesia was induced five to ten minutes after xylazine injection by mixture of ketamine hydrochloride (Anaket®, Richter Pharma, Austria) 2.2 mg·kg^{-1} with diazepam (Diazepam, Sopharma, Bulgaria) 0.05 mg·kg^{-1} injected intravenously in the two protocols. Trachea was intubated with 20 - 22 mm OD tracheal tube (Cook) and the horses were moved to operation theatre where were placed on a padded surgical table in dorsal recumbency.

Anaesthesia was maintained for 3 hours with isoflurane (Foran®, Abbot labretories, Switzerland) in oxygen 100% through closed circuit system of a large animal anaesthesia machine LDS 300 (Surgivet, USA) equipped with large animal ventilator DHV 1000 (Surgivet, USA) and out—of—circuit isoflurane vaporizer (Penion Limited Abington, Oxon, Ox 143 PH, UK). As soon as the vaporizer was switched on, group ISOD received a CRI of dexmedetomidine hydrochloride (Dexdomitor®, Orion Pharma, Finland) 1.75 µg·kg^{-1}·hour^{-1} diluted with saline to a concentration of 0.01 mg·ml^{-1}, while group ISO received a CRI of equivalent volume of saline solution administered by means of microinfusion pump WZ—50C6 (All Pro, China) until the end of anaesthesia. Syringes were prepared in advance so as the anaesthetist was unaware of medications given.

Monitoring was performed throughout anaesthesia using a patient monitor PM—9000Vet (Mindray, China). The main clinical parameters heart rate (HR), electrocardiogram (ECG), haemoglobine oxygen saturation (Sat), respiratory rate (RR), inspired (FiIso) and expired (EtIso) fractions of isoflurane, inspired and expired fractions of CO_2 ($FiCO_2$, $EtCO_2$), of O_2 (FiO_2, EtO_2), and minimal alveolar concentration (MAC) of isoflurane were recorded every five minutes. For ECG recording II lead was used derived from sternal—wither configuration. Saturation probe was placed on the tongue.

Systolic (SYS), diastolic (DIA), and mean (MEAN) arterial blood pressures were measured invasively after cannulation of left or right facial artery using 22—gauge 0.9 × 25 mm catheter (Venocan plus®, Kruuse, Denmark). Arterial blood samples were collected immediately after catheter placement and before the end of anaesthesia for blood gases, electrolytes and acid—base status measurement. Repiratory/blood gases VetStat® cassettes and VetStat® electrolyte and blood gas analyzer (IDEXX Laboratories, Inc., USA) were used for that purpose. Arterial lactate levels were measured by colorimetric method using enzymatic Roche/Hitachi lactate reagent (Roche Diagnostica, Germany).

Venous blood samples were taken at the beginning and at the end of anaesthesia in vials containing sodium citrate for measurement of some parameters of coagulation system such as fibrinogen, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT) using coagulometer Amelung KC1A (Germany) and tests of Human Diagnostica (Germany). Blood D-dimer was measured by means of quantitative latex—aglutination method (Spinreact, Spain).

Second venous catheter was used for administration of Ringer's solution (Actavis, Bulgaria) at a minimal rate of 10 ml·kg^{-1}·hour^{-1} with some corrections throughout the anaesthesia. The rate was adjusted to maintain mean arterial pressure above 60 mmHg. Nevertheless, if blood pressure continued to drop, dopamine hydrochloride (Warsaw Pharmaseutical Works Polfa SA, Poland) was infused starting with a rate of 0.5 µg·kg^{-1}·minute^{-1} and

adjusted as required.

The animals were allowed to breathe spontaneously. If the arterial partial pressure of CO_2 ($PaCO_2$) increased above 60 mmHg, the arterial partial pressure of O_2 (PaO_2) decreased bellow 100 mmHg, or RR was lower than 4 breaths minute^{-1} for more than 3 minute an intermittent positive pressure ventilation (IPPV) was provided. Tidal volume of 20 ml·kg^{-1}, peak inspiratory pressure (PIP) no more 30 cm H_2O, RR 8 breaths minute^{-1}, and inspiratory time 2.5 seconds were set in an assisted-controlled mode of respiration. Urinary catheter was placed as well.

Deep surgical plane of anaesthesia was maintained by altering the vaporizer setting and thus inspired isoflurane concentrations. The depth of anaesthesia was assessed observing RR, HR, blood pressures, ocular position, movements, reflexes and moisture. Moreover, nociceptive electrical stimulation was given 30 minutes after induction of anaesthesia and every 30 minutes thereafter. For that purpose two surface electrodes were applied to the shaved and moistured skin over the lateral palmar digital nerve between the coronary band and the fetlock joint of the right hind limb, 1 cm apart and secured with elastic bandage. A constant current (CC) electrical stimulation was delivered [19] with the aim of an electrical stimulator Tonus 2M (Drujba Ltd., Vratza, Bulgaria). In case of positive reaction to electrical stimulation (gross purposeful movement of non stimulated limbs) or spontaneous movement of the horse without stimulation the isoflurane concentration was increased.

Three hours after tracheal intubation, the vaporizer was switched off and the animals allowed to recover in a quiet padded box. Horses were extubated as soon as they were able to swallow. No any assistance, oxygen supplementation, or additional sedation was applied during this period. The quality of recovery was evaluated by recording extubation time, time to sternal and time to standing positions, and observing the number of attempts to stand, the presence of violence, ataxia, or injury. The quality of recovery was scored from 1 to 5 grades (**Table 1**).

2.3. Statistical Analysis

Data were analyzed by means of a commercially available software package (Statistica® 6-0 version, StatSoft Inc. USA). The distribution of continuous data was tested using the Kolmogorov-Smirnov test. Analysis of variance (ANOVA) for repeated measurements was used to detect the influence of time and treatment upon each anaesthesiological or clinical variable. Factorial ANOVA was used to test the alterations in blood gases, electrolytes, and haemocoagulation parameters. Fisher LSD post-hoc analysis was performed in order to determine the probability value. Recovery durations were compared between two anaesthetic protocols using one-way ANOVA and post-hoc Fisher test. Recovery scores were compared in the Wilcoxon signed rank test. The minimal level of statistical significance was set at 0.05 for all analyses.

3. Results

HR, RR, Sat, SYS, MEAN, and DIA remained unchanged throughout isoflurane anaesthesia alone, whereas RR and Sat decreased over time in ISOD group with statistically significant alterations at 150 and 180 minutes comparatively to the initial period. Comparative analysis between groups showed several periods with lower HR, higher RR, SYS and MEAN values in horses anesthetized with PIVA using dexmedetomidine and isoflurane in relation to isoflurane anaesthesia alone (**Table 2**). The absence of changes in the ECG was found in both anaesthesia types for the entire period tracked.

The inspired and expired fractions of oxygen remained high during the whole anaesthesia in two groups (**Table 3**). $EtCO_2$ was maintained in acceptable limits. The need for inhalation anaesthetic decreased over time assessed by Vol%, MAC, FiIso, and EtIso. Anaesthetic requirements were lower in ISOD protocol which was

Table 1. Assessment of recovery quality in anaesthetized horses.

Description	Points
Getting up after one attempt, without ataxia	1
Getting up after one or two attempts, mild ataxia	2
More than two attempts to get up, but quiet	3
More than two attempts to get up, agitation	4
Significant violence, self-injury	5

Table 2. Values (Mean ± SD) of the clinical parameters of horses subjected to isoflurane anaesthesia (group ISO) and combined isoflurane inhalation with CRI of dexmedetomidine (group ISOD) detected at 10 minute intervals.

parameter	group	n	30'	40'	50'	60'	70'	80'	90'	100'	110'	120'	130'	140'	150'	160'	170'	180'
HR, min^{-1}	ISO	6	40.0 ± 3.22	40.7 ± 11.4	38.7 ± 11.2	38.8 ± 9.0	38.3 ± 9.5	38.3 ± 6.7	39.0 ± 10.2	41.7 ± 7.0	41.2 ± 5.7	39.7 ± 6.6	40.7 ± 5.6	38.7 ± 4.3	39.5 ± 5.0	39.8 ± 5.2	40.2 ± 5.0	40.5 ± 4.1
	ISOD	6	36.2 ± 5.5	36.8 ± 4.7	35.2 ± 4.5	35.2 ± 4.4	35.7 ± 5.6	36.8 ± 6.2	38.7 ± 6.7	34.8 ± 6.5	34.8 ± 6.9$^{▲}$	34.5 ± 6.9	35.8 ± 6.4	34.8 ± 6.7	33.3 ± 5.0	31.3 ± 2.8$^{▲▲}$	31.5 ± 3.0$^{▲▲}$	34.0 ± 4.9$^{▲}$
RR, min^{-1}	ISO	6	7.3 ± 3.1	7.8 ± 3.7	7.8 ± 5.0	5.7 ± 1.9	5.7 ± 2.3	5.5 ± 1.8	5.5 ± 2.2	5.0 ± 0.9	6.0 ± 2.7	5.8 ± 3.2	5.5 ± 3.7	5.8 ± 3.1	5.7 ± 1.9	6.0 ± 2.7	6.5 ± 3.3	6.0 ± 2.7
	ISOD	6	10.8 ± 3.8	10.0 ± 2.5	8.7 ± 1.8	8.7 ± 2.6$^{▲}$	9.8 ± 5.6	7.7 ± 2.8	7.8 ± 2.1	8.3 ± 1.5$^{▲▲▲}$	7.7 ± 2.6	9.0 ± 2.0	9.0 ± 3.5	9.0 ± 4.1	7.8 ± 3.0*	9.5 ± 4.8	8.5 ± 3.6	7.8 ± 3.4*
Sat, %	ISO	6	94.8 ± 4.3	94.7 ± 6.3	95.3 ± 3.5	96.0 ± 3.3	96.2 ± 2.0	95.8 ± 2.9	95.8 ± 3.1	95.5 ± 2.7	95.8 ± 2.9	96.0 ± 3.5	96.8 ± 2.4	97.0 ± 1.5	94.8 ± 3.6	94.2 ± 3.5	95.7 ± 2.1	92.8 ± 4.9
	ISOD	6	96.0 ± 2.5	96.2 ± 1.0	96.0 ± 1.8	95.7 ± 2.6	95.3 ± 1.5	93.0 ± 3.8	93.7 ± 4.2	92.2 ± 4.6	92.7 ± 3.9	92.2 ± 5.3	91.3 ± 5.8	91.0 ± 6.8	90.0 ± 6.1	89.3 ± 6.5	90.3 ± 7.3	88.7 ± 4.8*
SYS, mmHg	ISO	6	78.5 ± 28.7	82.3 ± 32.2	74.3 ± 20.6	76.2 ± 18.9	77.2 ± 23.5	76.3 ± 19.6	77.3 ± 21.5	80.5 ± 15.5	81.3 ± 11.0	81.3 ± 9.7	80.5 ± 9.2	88.5 ± 15.6	89.8 ± 10.7	85.5 ± 13.5	86.0 ± 11.7	79.8 ± 7.4
	ISOD	6	100.3 ± 12.5	100.2 ± 15.5	87.0 ± 12.7	76.7 ± 25.3	92.7 ± 14.0	88.7 ± 5.1*	95.0 ± 6.1	89.8 ± 10.3	91.3 ± 14.1	94.5 ± 6.9$^{▲}$	101.8 ± 5.6$^{▲▲▲}$	99.7 ± 8.4	99.2 ± 14.3	97.8 ± 10.1	97.7 ± 10.3	95.5 ± 8.8$^{▲▲}$
MEAN, mmHg	ISO	6	60.3 ± 27.4	61.7 ± 27.0	54.8 ± 13.8	55.3 ± 12.4	59.7 ± 17.7	57.5 ± 14.2	63.2 ± 16.7	62.5 ± 14.0	64.0 ± 11.6	65.0 ± 9.1	65.0 ± 8.6	67.2 ± 12.1	67.7 ± 9.0	62.8 ± 11.3	67.2 ± 11.2	63.2 ± 10.3
	ISOD	6	68.7 ± 10.8	68.5 ± 14.3	64.0 ± 3.1	63.3 ± 5.8	72.2 ± 13.3	67.3 ± 9.5	70.7 ± 13.5	68.3 ± 9.6	71.7 ± 8.2	73.7 ± 12.4	78.0 ± 11.3$^{▲}$	77.7 ± 10.5	76.0 ± 11.3	77.0 ± 15.0	76.0 ± 14.9	73.5 ± 14.5
DIA, mmHg	ISO	6	48.7 ± 25.3	49.3 ± 28.6	46.3 ± 11.5	46.3 ± 12.0	52.7 ± 17.3	48.2 ± 13.0	53.0 ± 13.8	51.0 ± 13.2	52.0 ± 11.2	55.3 ± 10.7	55.5 ± 10.2	55.0 ± 10.5	53.3 ± 8.3	50.2 ± 9.7	52.3 ± 10.6	52.7 ± 12.4
	ISOD	6	52.5 ± 11.4	53.5 ± 14.6	51.5 ± 9.4	52.3 ± 9.3	61.3 ± 15.9	53.0 ± 14.2	54.7 ± 16.2	53.3 ± 7.3	57.7 ± 4.7	57.0 ± 13.7	61.3 ± 13.4	59.2 ± 13.6	58.2 ± 13.0	57.0 ± 13.3	58.0 ± 9.8	56.7 ± 11.1

$^{*}p < 0.05$; —level of significance of differences in relation to the initial period. $^{▲}p < 0.05$; $^{▲▲}p < 0.01$; $^{▲▲▲}p < 0.001$ level of significance between two anaesthetic types in one and the same period.

Table 3. Values (Mean ± SD) of the anaesthesiological parameters of horses subjected to isoflurane anaesthesia (group ISO) and combined isoflurane inhalation with CRI of dexmedetomidine (group ISOD) detected at 10 minute intervals.

Parameter	group	n	30’	40’	50’	60’	70’	80’	90’	100’	110’	120’	130’	140’	150’	160’	170’	180’
$EtCO_2$, mmHg	ISO	6	41.5 ± 10.3	43.5 ± 12.8	37.0 ± 11.5	41.7 ± 10.6	43.8 ± 8.7	44.3 ± 8.6	45.3 ± 9.1	42.3 ± 9.3	47.2 ± 7.7	45.2 ± 8.3	46.7 ± 10.6	44.5 ± 11.6	43.0 ± 13.6	45.7 ± 12.7	45.3 ± 11.1	43.2 ± 12.7
	ISOD	6	40.5 ± 15.9	41.3 ± 11.1	41.7 ± 14.4	39.5 ± 13.3	45.0 ± 9.6	45.8 ± 13.5	44.3 ± 13.4	40.8 ± 6.6	45.2 ± 11.1	40.3 ± 6.6	43.7 ± 7.6	42.0 ± 7.8	41.7 ± 13.0	40.7 ± 13.3	41.7 ± 15.1	43.2 ± 17.0
FiO_2, %	ISO	6	88.8 ± 4.0	90.2 ± 3.2	91.8 ± 5.1	89.3 ± 6.8	92.4 ± 5.7	91.9 ± 5.0	92.4 ± 3.7	91.2 ± 4.4	90.5 ± 5.5	92.3 ± 3.4	91.8 ± 3.9	91.9 ± 4.4	91.2 ± 5.5	90.7 ± 4.0	92.3 ± 3.0	93.5 ± 3.9
	ISOD	6	86.6 ± 12.4	90.3 ± 5.0	92.5 ± 3.1	91.7 ± 5.3	93.0 ± 3.9	92.5 ± 3.9	92.0 ± 4.8	92.2 ± 4.9	93.5 ± 2.6	92.0 ± 4.7	91.1 ± 4.9	93.3 ± 3.8	92.1 ± 4.1	88.2 ± 10.6	90.3 ± 3.7	93.1 ± 2.5
EtIso, %	ISO	6	1.57 ± 0.34	1.48 ± 0.34	1.44 ± 0.26	1.40 ± 0.17	1.38 ± 0.05	1.35 ± 0.07	1.39 ± 0.08	1.34 ± 0.07	1.35 ± 0.07	1.29 ± 0.08	1.29 ± 0.08	1.29 ± 0.08	1.27 ± 0.08	1.27 ± 0.13	1.28 ± 0.09	1.29 ± 0.08
	ISOD	6	0.97 ± 0.17$^{▲▲▲}$	0.94 ± 0.12$^{▲▲▲}$	0.96 ± 0.29$^{▲▲}$	1.05 ± 0.27$^{▲▲}$	1.15 ± 0.21$^{▲}$	1.23 ± 0.15	1.15 ± 0.12$^{▲}$	1.07 ± 0.29$^{▲}$	1.01 ± 0.32$^{▲}$	1.05 ± 0.15$^{▲}$	0.96 ± 0.25$^{▲▲}$	0.96 ± 0.18$^{▲▲}$	0.84 ± 0.2$^{▲▲▲}$	0.85 ± 0.2$^{▲▲}$	0.84 ± 0.15$^{▲▲▲}$	0.86 ± 0.10$^{▲▲▲}$
FiIso, %	ISO	6	1.72 ± 0.44	1.69 ± 0.53	1.74 ± 0.38	1.69 ± 0.3	1.62 ± 0.28	1.53 ± 0.19	1.57 ± 0.32	1.56 ± 0.24	1.57 ± 0.22	1.50 ± 0.21	1.5 ± 0.15	1.5 ± 0.14	1.43 ± 0.15	1.42 ± 0.21	1.42 ± 0.2	1.46 ± 0.15
	ISOD	6	1.17 ± 0.5	1.13 ± 0.34	1.0 ± 0.32$^{▲}$	1.08 ± 0.28$^{▲}$	1.54 ± 0.25	1.68 ± 0.32	1.38 ± 0.21	1.29 ± 0.34	1.17 ± 0.29	1.23 ± 0.18	1.08 ± 0.22$^{▲}$	1.08 ± 0.17$^{▲▲}$	0.93 ± 0.15$^{▲▲}$	0.94 ± 0.17$^{▲▲}$	0.93 ± 0.13$^{▲▲}$	1.0 ± 0.17$^{▲▲}$
MAC, %	ISO	6	1.13 ± 0.3	1.18 ± 0.26	1.15 ± 0.2	1.13 ± 0.16	1.07 ± 0.16	1.07 ± 0.15	1.1 ± 0.13	1.05 ± 0.08	1.1 ± 0.11	1.08 ± 0.08	1.08 ± 0.1	1.05 ± 0.1	1.05 ± 0.1	1.02 ± 0.13	1.03 ± 0.12	1.07 ± 0.1
	ISOD	6	0.93 ± 0.38	0.9 ± 0.21	0.85 ± 0.21$^{▲}$	0.87 ± 0.22$^{▲}$	1.03 ± 0.12	1.13 ± 0.16	1.0 ± 0.11	0.98 ± 0.17	0.93 ± 0.23	1.02 ± 0.13	0.92 ± 0.19	0.93 ± 0.15	0.82 ± 0.15$^{▲}$	0.83 ± 0.16	0.82 ± 0.12$^{▲}$	0.87 ± 0.18$^{▲}$
Vol, %	ISO	6	4.83 ± 0.41	4.25 ± 0.76	3.67 ± 0.61**	3.25 ± 0.52**	3.17 ± 0.26***	3.0 ± 0.45***	2.92 ± 0.38***	2.83 ± 0.26***	2.83 ± 0.26***	2.83 ± 0.26***	2.83 ± 0.26***	2.58 ± 0.38***	2.58 ± 0.38***	2.75 ± 0.27***	2.67 ± 0.26***	2.67 ± 0.27***
	ISOD	6	2.83 ± 0.41$^{▲▲▲}$	2.42 ± 0.8$^{▲▲}$	1.9 ± 0.6$^{**▲▲}$	1.9 ± 0.6$^{**▲▲}$	2.12 ± 0.51$^{▲▲}$	2.02 ± 0.68$^{▲}$	1.83 ± 0.59$^{*▲▲}$	1.67 ± 0.39$^{**▲▲▲}$	1.58 ± 0.29$^{**▲▲▲}$	1.65 ± 0.53$^{**▲▲}$	1.45 ± 0.39$^{***▲▲▲}$	1.43 ± 0.41$^{***▲▲}$	1.28 ± 0.34$^{***▲▲▲}$	1.37 ± 0.45$^{***▲▲▲}$	1.4 ± 0.42$^{***▲▲▲}$	1.43 ± 0.41$^{***▲▲▲}$

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ —level of significance of differences in relation to the initial period. $^{▲}p < 0.05$; $^{▲▲}p < 0.01$; $^{▲▲▲}p < 0.001$ —level of significance between two anaesthetic types in one and the same period.

more pronounced at final periods. The mean reduction of MAC was calculated to be 15.6%, EtIso was decreased by 23% in relation to ISO group. The drop in Vol% was even more pronounced.

Both isoflurane and isoflurane-dexmedetomidine anaesthesias produced insignificant respiratory acidosis after 3 hours duration (**Table 4**). PaO_2 significantly decreased in ISOD group comparatively to the initial values. At final period blood lactate levels increased in iso-dex anaesthesia in relation to the 0 period as well as to the isoflurane anaesthesia alone.

Coagulation parameters did not changed during both anaesthesia types with the exception of slightly higher fibrinogen levels in ISOD relatively to the ISO group at 3rd hour (**Table 5**).

In terms of times and quality of recovery we did not find significant differences between the two groups (**Table 6**).

4. Discussion

According to the results of Marcilla *et al.* [16] aconstant rate infusion of dexmedetomidine produced similar cardiovascular effects in isoflurane anaesthetized horses independent of infusion rates used (1 and 1.75 $\mu g \cdot kg^{-1} \cdot hour^{-1}$). We used the second rate as it is considered equipotent to CRI of the other alpha-2 agonists described in the introduction section [20]. This rate of dexmedetomidine used in our study renders enough to

Table 4. Blood gases and acid-base (BG-AB) parameters of horses (Mean ± SD) subjected to isoflurane anaesthesia (group ISO) and combined isoflurane inhalation with CRI of dexmedetomidine (group ISOD).

Parameters	group ISO (n = 6)		group ISOD (n = 6)	
	0 Hour	3 Hour	0 Hour	3 Hour
pH	7.418 ± 0.07	7.317 ± 0.117	7.428 ± 0.054	7.315 ± 0.06
$PaCO_2$, mmHg	50.0 ± 12.47	71.33 ± 19.37	45.0 ± 6.2	63.83 ± 20.55
HCO_3, mmol/l	29.18 ± 4.53	32.68 ± 2.25	27.20 ± 1.87	29.23 ± 4.8
tCO_2, mmol/l	30.70 ± 4.79	34.85 ± 2.67	28.6 ± 1.96	31.2 ± 5.39
BE, mmol/l	4.33 ± 3.37	5.13 ± 1.53	2.87 ± 1.96	1.75 ± 3.24▲
PaO_2, mmHg	175.33 ± 59.17	134.5 ± 51.07	187.17 ± 34.25	92.33 ± 26.16***
tHb, g/l	85.33 ± 12.45	80.17 ± 17.24	97.17 ± 27.18	107.5 ± 37.0
Sat, %	98.83 ± 1.47	97.17 ± 2.32	99.0 ± 0.89	93.17 ± 4.79
Na^+, mmol/l	143.83 ± 4.58	144.17 ± 3.06	140.67 ± 3.5	142.0 ± 7.59
K^+, mmol/l	4.17 ± 0.8	3.68 ± 0.47	4.48 ± 0.77	3.53 ± 0.51
Cl^-, mmol/l	107.0 ± 2.53	106.67 ± 2.5	106.33 ± 1.97	105.17 ± 6.68
Lactate, mmol/l	1.16 ± 0.49	1.52 ± 0.64	1.64 ± 0.86	3.03 ± 1.23*▲
AG, mmol/l	11.97 ± 3.16	8.45 ± 1.07	11.63 ± 1.95	11.0 ± 3.25

*$p < 0.05$; ***$p < 0.001$—level of significance of differences between two periods. ▲$p < 0.05$—level of significance of differences between two anaesthesia types for the respective period.

Table 5. Blood coagulation parameters of horses (Mean ± SD) submitted to isoflurane anaesthesia (group ISO) and combined isoflurane inhalation with CRI of dexmedetomidine (group ISOD).

Parameters	Group ISO (n = 6)		Group ISOD (n = 6)	
	0 hour	3 hour	0 hour	3 hour
Фибриноген, g/l	2.85 ± 0.80	2.34 ± 0.75	3.67 ± 0.28	3.44 ± 0.64▲
APTT, s	53.05 ± 10.23	54.83 ± 11.68	57.68 ± 10.38	65.68 ± 9.02
PT, s	11.78 ± 0.84	13.3 ± 1.86	14.42 ± 1.31	15.35 ± 5.06
TT, s	18.70 ± 2.53	20.17 ± 1.66	18.93 ± 3.43	21.10 ± 7.94
D-dimer, µg/ml	1.40 ± 0.62	1.86 ± 0.99	0.97 ± 0.55	2.51 ± 1.92

▲$p < 0.05$; level of significance of differences between two anaesthesia types for the respective period.

Table 6. Recovery score and times in isoflurane anaesthetized horses with (group ISOD) and without (group ISO) a CRI of dexmedetomidine.

Parameters	Group ISO (n = 6)	Group ISOD (n = 6)
Score (Mean ± SD)	2.17 ± 0.75	1.67 ± 0.52
Score Median (IQR)	2 (2 - 3)	2 (1 - 2)
Time of extubation, min.	8.0 ± 2.45	7.33 ± 2.58
Time for sternal recumbancy, min.	18.5 ± 8.26	21.0 ± 4.34
Time for standing, min.	41.17 ± 9.15	38.33 ± 6.15

reduce isoflurane requirements for maintenance of surgical anaesthesia, but the degree of reduction was lower than expected. The reason might be the lack of loading dose of dexmedetomidine in our protocol where it was replaced by xylazine premedication. Another possible cause could be the low CRI used to supplement the inhalation agent. The way of calculation of MAC reduction was not uniform in all investigations which might result in different percentage of decrease in volatile agent concentration during PIVA. The drop in isoflurane needs was much more pronounced when PIVA was combined with CRIs of other agents such as lidocaine, midazolam, or ketamine [21] [22]. Lidocaine proved to decrease MAC of volatile agents dose-dependantly but leads to ataxia during recovery period. Ketamine also has potentiated inhalation anaesthesia but the occurrence of excitation during recovery could not be prevented. Therefore, alpha-2 agonists remained the agents of choice for PIVA.

We observed stable cardiovascular function during both isoflurane and isoflurane-dexmedetomidine protocols with several periods of elevated systolic and mean arterial pressures in ISOD group. This difference probably was connected with the effect of dopamine infusion to maintain blood pressures above safe limits. Horses anaesthetized with isoflurane-dexmedetomidine received the total of 35 mililitres, whereas horses from ISO group were given 30 mililiters of dopamine. HR was lower compared to ISO group, a typical side effect of alpha-2 agonists that was reported by other authors [17] [21].

Although all animals were breathing 100% of oxygen and the lowest inspired and expired fractions of oxygen were 86.6% and 79.0%, respectively, we found out gradually drop in PaO_2 that was more obvious in ISOD group. This effect has been reported by other investigators [17] and resulted in elevated lactate levels. The respiratory function in horses is altered by anaesthetic drugs and bodyposition especially during dorsal recumbency. A reduction of PaO_2 is caused by diffusion abnormalities, ventilation-perfusion mismatching or right to left shunts independent on 50% or maximal inspired oxygen concentration [23], whereas $PaCO_2$ is rarely elevated under these conditions because of the stimulation of ventilation as a result of hypoxaemia [24]. The $EtCO_2$ and $PaCO_2$ correlate positively but in horses $EtCO_2$ tends to be 10 to 15 mmHg lower than $PaCO_2$ [25]. IPPV was necessitated in one horse from ISO group and three horses in ISOD group. Hypoventilation is a common finding during equine anaesthesia because virtually all drugs cause respiratory depression. The increase in $PaCO_2$ during isoflurane anesthesia is attributable to a decrease in respiratory rate, whereas tidal volume is maintained or increases [26]. Horses anesthetized with isoflurane in 100% oxygen often have an irregular ventilatory rhythm, characterized by a low respiratoryrate. The addition of dexmedetomidine CRI to isoflurane increased RR but did not improved ventilation and oxygenation.

Changes in coagulation system during general anaesthesia in horses were rarely investigated. Inhalation anaesthetics inhibit thrombocyte aggregation at MAC above 1. However, this effect did not result in alteration of measured coagulation parameters. Our results are in accordance with the study of Elrashidy *et al*. [27] where isoflurane did not have any effect either on thrombocyte aggregation or on the rest of coagulation parameters. The difference in fibrinogen values between groups at 3rd hour resulted from slight decrease in isoflurane anaesthesia.

For equine anaesthesia, 25% - 50% of fatalities are a direct result of injury sustained during recovery [1] [28]. In addition to the horse's physical condition and temperament, the environment at the recovery site and the type of surgery, the quality and time of recovery is related to the dose and route of anaesthetic drug administration, the duration of anaesthesia, the cardiopulmonary function during maintenance of anaesthesia and the administration of sedatives or drug antagonists during the recovery period [1]. A challenge for the anaesthetist is to find a dosing regimen that provides adequate anaesthetic depth and ensures that horses regain sufficient strength and coordination before they try to stand up. Our experimental horses showed good quality of recovery with similar scores for both isoflurane anaesthesia alone and isoflurane-dexmedetomidine without any sedation, oxygen sup-

plementation, or assistance. Times for extubation, for sternal and standing positions also were similar between groups. Therefore, dexmedetomidine CRI did not influence the time and quality of recovery during isoflurane anaesthesia in healthy horses which was opposite to the data of Marcilla *et al*. [17] who reported better but longer recovery when dexmedetomidine was added to isoflurane.

5. Conclusion

In conclusion, the use of dexmedetomidine CRI as an anesthetic adjunct to isoflurane anaesthesia reduced slightly isoflurane requirements, maintained stable cardiovascular function, produced greater respiratory depression than isoflurane alone, and had no effects on haemocoagulation as did isoflurane alone. Recovery times and quality were good and comparable between groups.

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End of Life Care Medical Education: 48 Hour Hospice Home Immersion Alters Students' Lives

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Abstract

Introduction: Education and skill enhancement in palliative and end of life care is rarely part of the foundational medical education curriculum. The progress of student physicians tends to be measured by their ability to synthesize and demonstrate basic medical knowledge and clinical skills but offers little assessment of the maturation of attitudes or their values. The University of New England College of Osteopathic Medicine (UNECOM), immerses second year medical students in a hospice home for 48 hours to enhance students' perspectives in interprofessional palliative and end of life care. Methods: This project utilized qualitative ethnographic and autobiographic research designs. Two female second year medical students (27 y/o & 26 y/o) were immersed for 48 hours into a local hospice home, sleeping in a bed where others had died, to answer the question: "*What is it like for ME to live in the Hospice Home for 48 hours and how does this contribute to my future as a practitioner*?" Data were collected in the form of journal notes for pre-fieldwork, fieldwork, and post-fieldwork and included subjective and objective reporting of observations, experiences, and patient/family encounters. Analyses included journal review and thematic categorization and coding through content analysis. Results: Themes common to both students that factored in the research question and their prior stated interest areas of medical humanities and person-centered care at end of life were identified. Three themes were selected for this article: 1) Person-Centered Experiences, 2) Spectrum of Communication, and 3) Introspection: Attitudes and Values. The process of living in the hospice home for 48 hours revealed students' attitudes about various disease processes, their personal experiences with death and dying, and their assumptions about how patients approach death. Conclusion: This *Hospice Home Immersion* project provided both an educational approach and learning environment that was effective in advancing medical students' attitudes, skills, and knowledge as evidenced by their self-reported life altering

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learning about end of life and palliative care.

Keywords

End of Life Care, Palliative Care, Hospice Home, Medical Education, Immersion Learning

1. Introduction

The pre-clinical years of medical school (year 1 and year 2) are focused on recognizing and understanding disease; however education and skill enhancement in palliative and end of life care is rarely part of the foundational curriculum. The progress of student physicians during this time is measured by their ability to synthesize and demonstrate basic medical knowledge and clinical skills but offers little assessment of the maturation of attitudes or their values. The attitudes that medical students harness from early childhood through their participation in medical school affect their philosophy about end of life care and the development of good care provision to dying patients and their families [1].

A novel approach to expanding student physician exposure to palliative and end of life care and broadening student perspective through experiential education has been developed at the University of New England College of Osteopathic Medicine (UNECOM). Second year medical students volunteered to pair up for a 48-hour continuous immersion in a hospice home to participate in inter-professional palliative and end of life care. This educational approach and learning environment have been found effective in advancing attitudes, skills, and knowledge for these medical students [2]. The experiences of two students, who conducted this research project, illustrate their pathways for advanced and life-altering learning about end of life and palliative care.

1.1. End of Life Care in Early Medical Education

In 1997, the Institute of Medicine published its landmark report: *Approaching Death*: *Improving Care at the End of Life*, in which it noted that "the education and training of physicians and other health care professionals fail to provide them the attitudes, knowledge, and skills required to care well for the dying patient" [1] [2]. The medical education community has a responsibility to provide, at the least, competent care for dying patients. Deficiencies in educating future physicians in end of life care reflect a medical culture that defines death as failure and ignores care for dying people as a source of professional accomplishment and personal meaning [1]. Major deficiencies in the end of life medical education include: 1) a curriculum in which death is conspicuous mainly by its relative absence; 2) educational materials that are notable for their inattention to the end stages of most diseases and their neglect of palliative strategies; and 3) clinical experiences for students and residents that largely ignore dying patients and those close to them [1].

Today, nearly all United States medical schools offer some integration of death and dying in the required curricula, but dedicated courses in end of life care exist in fewer than 30% of schools [3] [4]. Additionally, the dedicated content on this topic is offered through intermittent lectures, case studies/problem-based learning, and possibly brief preceptorships, all of which fail to provide any depth or extended bedside experiences to augment knowledge, skills and attitudes in palliative medicine [5].

Too many first and second year medical students have heard the challenging stories from their peers in third and fourth year clerkships of violent hospital deaths, the discomfort exhibited by attending physicians when they need to address terminal illness with their patients, and the dearth of discussion on advance care planning and options for palliative treatments for patients and their families [6]. Third and fourth year medical students report that they seldom have the opportunity to participate in the care of dying patient and if they do, there is no protected space to reflect or time to formally debrief the situation [7].

Many physicians are remiss in communicating the option of either home hospice (care provided in the person's home) or hospice home care (care provided in an environment dedicated to end of life care) for their patients. An article published in the journal *Cancer*, reported that when 4074 physicians surveyed were asked about the treatment of a cancer patient with four to six months to live, just 26 percent of the physician respondents indicated they would initiate a conversation about hospice, with 49 percent selecting that they would delay a hospice discussion [8].

Such issues are of critical importance to address in medical education and training. The medical school environment has a social responsibility to prepare future physicians especially in the field of end of life and palliative care; no person will avoid death. Medical students, who are unaware of or have unexamined personal anxieties about death, may unconsciously distance themselves from those who are dying. If not explored and understood, this could, in the future, ultimately fail their patients clinically and emotionally [9] [10].

1.2. Project Inception

A group of UNECOM students expressed a profound interest in medical humanities and person-centered care; two philosophies that may be best learned in the field. They questioned how they could advance their education to include these practice philosophies that are intimately tied to health and health care with end of life care. These students held the belief that the state-of-mind of a person (who may be a patient) has as profound effect on disease as well as on the body itself. Additionally, they believed that the attitudes and values of a physician shape the person (patient)-physician relationship, which impacts the course of care. These premises framed their 48-hour immersion into the Hospice Home.

1.3. 48 Hour Hospice Home Immersion Defined

The Learning by Living 48 Hour Hospice Home Immersion Project (referred to as *Hospice Home Immersion*) was piloted in December, 2014 [10]. It was designed and implemented as an experiential medical education learning model by the Director of Geriatrics Education and Research within the Medical School's Department of Geriatric Medicine, who is also the project principal investigator (PPI). The *Hospice Home Immersion* project is based on the long standing and highly successful UNECOM Learning by Living Nursing Home Immersion Project in which medical students and other health professions students are "admitted" into nursing homes to live the life of an older nursing home resident for approximately two weeks—24 hours a day/7 days a week—complete with medical diagnoses and "standard" procedures of care [11] [12]. However, medical students who participate in the *Hospice Home Immersion* project are active participants in patient (who die on average within 4.5 days of arriving in the home), family, and post-mortem care during 48 continuous hours rather than assuming the role of "patient or resident" [5]. The purpose of the *Hospice Home Immersion* project is to provide second through fourth year medical students with firsthand experiences of living in the Hospice of Southern Maine (non-profit) Gosnell Memorial Hospice Home, Scarborough, ME, to answer the question: "*What is it like for ME to live in the Hospice Home for 48 hours and how does this contribute to my future as a practitioner*?" [5].

2. Methods

The *Hospice Home Immersion* project utilized ethnographic and autobiographic research designs, whereby a unique environment or "culture" (Hospice Home) was observed and the life experiences of the medical student before, during, and immediately after the immersion were reported [5]. As this was conducted with qualitative research methods, students' assumptions were identified rather than hypotheses and there are no variables associated with quantitative research. Written assumptions were based on those thoughts and feelings that each student identified and either accepted as true or believed were certain to happen, without proof. For this article student assumptions were written about what it will be like to live in a hospice home, what to expect from hospice and end of life care, and how each may deal with death. Awareness of these, prior to being immersed, acts as an alert for the students to be aware of and "test" throughout their immersion in the environment.

Two second year medical students were immersed in the Hospice Home for 48 hours at different times. One female student, 27 years old, was immersed in February 2015 and the second female student, 26 years old, was immersed in July 2015. In both immersions, each student selected a fellow classmate to conduct the project with. Students are in good academic standing and volunteer to conduct the project. They completed a registration form that included a written statement about their interest in being immersed for 48 hours and their assumptions. They then met with the project principal investigator (PPI) for an orientation and discussed information about the in-patient hospice home, the research components, the inter-professional staff, and project details for student implementation. Upon being immersed in the Hospice Home, the students participated in a one-hour orientation to the home by the nurse manager [5]. Each student received a name tag and security tag that provided access to all secure areas except the room where drugs were stored. The students were then each paired with a certified

nursing assistant and then a nurse, eventually working with the physician, chaplain, social worker, volunteers and also on their own throughout the 48 hours. They answered call bells, assisted staff with patient care, engaged with patients and family members, provided post mortem care following a death and participated in inter-professional staff team meetings. At night, they retired to a room set up for patient care and slept in a bed where others have died before [5]. This project was exempt from IRB approval as the student researchers were collecting data focused on self-reflection (autobiographic). The Hospice of Southern Maine Ethics Committee approved the implementation of the UNECOM 48 Hour Hospice Home Immersion project [5].

Data and Analyses

Data were collected in the form of written journals, in which the students' documented thoughts and feelings during the three stages of ethnographic research: pre-field work (three days prior to entry), field work (during the immersion), and post fieldwork (5 - 7 days after discharge) [5]. Written journals were reviewed and reflected upon at least twice by the students and the Project Principal Investigator (PPI) to identify themes and determine agreed upon definitions for those themes. Then a step by step deductive formulation of content from each journal (representative quotes) were categorized within the appropriate identified themes. Revision of themes and associated content continued throughout the analyses to ensure reliability using formative and summative checks such as reflection on their own experiences with death or their own personality traits. Interpretation of thematic and content analyses culminated in collective final results for the two students [5] [13].

3. Results

A combination of their respective results, supported by content that were significant to both students that factored in the research questions and their aforementioned areas of interest of medical humanities and person-centered care are presented. Three themes meeting these criteria were selected: 1) Person-Centered Experiences, 2) Spectrum of Communication, and 3) Introspection: Attitudes and Values. Presented below, in their voices, were the experiences and resultant reflections that instilled learning.

3.1. Theme 1. Person-Centered Experiences

"*There was one patient that I had who was a younger woman. She was grasping at the air and trying to push it away. In the hospital, that would be seen as a delusion and would have been heavily medicated. But I asked her, what do you see*? *She saw all of these people who she knew, but she was trying to push them aside. She wanted her dad. She was trying to get to her dad. That was what she was seeing in her dying process… Terry* (*pseudonym*), *the hospice nurse, told me that what patients see when they are dying may not be real to us, but it is real to them. It is their reality*". (*C. Farrell, OMS III*, 2015)

"*Nancy* (*pseudonym*) *said on several occasions prior to her death that* '*she wasn't ready to go*'. *She was concerned because her husband would no longer be receiving her social security check and could not afford basic living expenses, she had* '*so many people she loved that she wasn't able to say goodbye to*', *and she didn't know who would take care of her family. The day Nancy died she had a terrible pain crisis that took some time to control. The staff told me that this often happened when someone was going through existential pain. When she died, though, she was relieved of the physical pain, but the existential pain was still present*". (*J. Kodela, OMS III*, 2015)

Our first instincts were to think about the physical symptoms of our respective patients; to establish a differential diagnosis for delusions or for pain. It took further reflection to realize that the key was not to label this behavior as abnormal and identify an etiology, but instead to acknowledge the behavior as an expression of the person's state-of-mind. It was evident from living in the Hospice Home for 48 hours that there were aspects of pain and suffering that escaped the traditional understanding of physiology that we learned during two years of medical school. On a number of occasions, we encountered various patients with uncontrollable pain, despite traditional management and high doses of analgesics. Over time and with information shared by the hospice staff we began to understand that not all symptoms are physiologically based. There are psychological manifestations that may go beyond medical understanding. The term that was used at the hospice home was "dysfunctional dying".

"*It* (*dysfunctional dying*) *is usually in patients who had a tough life, she* (*hospice nurse*) *explained, such as*

substance use or sexual or physical abuse. These patients have so much pain from their life that they cannot die peacefully. They feel agitated, get out of bed, are looking for something but don't know what. This is real pain. That existential pain that is manifested as physical pain. It is their spiritual dysfunction manifested as physical disturbance". (*C. Farrell, OMS III*, 2015)

This concept was something new and foreign and certainly not taught in our medical school. Experiencing and observing different forms of dying helped in understanding the holistic nature of disease, specifically the influence of the mind and spirit on the body. Although this is a concept that osteopathic medicine embraces, as students, we were not able to explore it in relation to palliative care and the dying process through our curriculum. It was made evident during our time in the Hospice Home how the contribution of person-centered state-of-mind affects overall health, and appears to be magnified during end of life care.

3.2. Theme 2. Spectrum of Communication

"*Greta* (*pseudonym*) *is clearly awake and responsive, but has lost her speech. She looks straight at you when you talk to her. She sits up in bed clutching her cross. I wonder if she knows where she is and what has happened to her. I wonder if she is scared. I wonder if she is happy that I am there*". (*C. Farrell, OMS III*, 2015)

"*I realized that I am someone who typically relies on conversation. I'm uncomfortable with silence. In the more intimate experiences I had with families, I couldn't rely on conversation or even find meaningful words. This left me with silence. Though I didn't initially pick up on that as part of the source of my discomfort, I realized that I became much more relaxed when I acknowledged eye contact and body language as a source of communication*". (*J. Kodela, OMS III*, 2015)

Communication goes far beyond words. We questioned when writing our pre field-notes, prior to entering the Hospice Home, whether we would know what words to say to patients or their family. Feelings of "uneasiness" and being "unequipped" were deeply felt and we both wrote about this in our journals. Pre-clinical medical education did not prepare us for these difficult conversations nor did we feel that role playing or reading about how to communicate with a dying person and their family would have prepared us for the myriad of issues that arose during the dying process or at the time of death while in the home. We learned quickly that dying is complicated and complex for some, not everyone goes peacefully. Though we felt well prepared to express ourselves, in that we believe ourselves to be articulate communicators, the value of spoken words was depreciated within the hospice setting. We realized that communication can be insignificant or imprudent when one only relies on spoken words. Upon reflecting on our experiences we realized how much we read body language and listened to what was expressed beyond words.

"*I kept thinking about how language can fail us. The conversation with Rachel* (*pseudonym*), *the hospice chaplain really stuck with me. I thought about how terribly misplaced the words would have been had I told a family like Nancy's 'she's at peace'. It's a silly assumption to think that everyone who has passed is completely at peace*". (*J. Kodela, OMS III*, 2015)

In this situation, the patient had been tormented by unresolved life experiences. Though she had passed, these concerns lingered or were not laid to rest. Colloquialisms like "at peace" and "in a better place" are often offered to the family, but their use can be unfitting without familiarity of the dying person and his/her family. In turn, we realized that communication through a quiet presence or nonverbal exchange was invaluable and respected and honored the person and the family. We arrived at the conclusion that effective communication is much more than words, and is a pillar of quality health care.

"*Communication should be the number one priority of all healthcare settings. I think we learn this to a certain extent in medical school and we attempt to improve our communication among inter-professional teams. I think hospice does a great job with this. But I hadn't thought as much about the barriers to communication with patients, especially those who have lost their ability to speak*". (*C. Farrell, OMSIII*, 2015)

Effective and meaningful communication at the end of life or through the palliative process relies on internal congruence, meaning that the three aforementioned elements (words, voice tone, and body language) support each other. The *Hospice Home Immersion* raised our communication consciousness and emphasized the importance of cultivating the tools necessary to mindfully communicate through action, tone, and words.

3.3. Theme 3. Introspection: Attitudes and Values

The *Hospice Home Immersion* was emotional. We each carried our own familiarity with death and dying, which

impacted the way in which we each responded to varying events in the hospice environment.

"*I am afraid that I will be too emotional, because I know that lying just beneath the surface are my own painful memories. I do not want to inflict my pain and suffering on someone else, most of all someone that I am trying to help and learn from. But I also want to maintain my humanity… I am still learning how to walk the line of being compassionate and professional*". (*C. Farrell, OMS III*, 2015)

Self-assessment and reflection were prominent themes throughout our journaling process and aided in advancing our learning about medical humanities and person-centered care. At times during the immersion, this reflection revealed unexpected biases. I was part of a discussion about a person dying from alcoholic cirrhosis. This patient had left home at an early age and only reached out to family in times of crisis. At the close of this encounter I thought long and hard about what I was thinking and feeling, finally I wrote:

"*His story didn't impact me immediately, until Rachel* (*pseudonym*), *the hospice chaplain, said 'Isn't his story inspiring*? *I wouldn't be able to survive homeless for 22 years. Just a really powerful story*'. *I realized in that moment that I was already becoming the* (*student*) *physician that I loathed. As I heard the details of his case, I had immediately felt badly for the family and judged Don* (*pseudonym*). *I had thought that it must have been terrible for the family to watch Don make so many bad decisions and wondered why he had so much trouble. Though I hadn't voiced any of this, I was instantly embarrassed by my thoughts. The discussion with Rachel was incredibly valuable and grounding*". (*J. Kodela, OMS III*)

There were times that our assumptions about patient desires during end of life care were misguided.

"*Sometimes as providers you make assumptions about what the patient wants. I stayed up with Diana* (*pseudonym*) *all night, thinking that I did not want her to die alone. This morning I learned that she had died about an hour after I went to bed. I felt pretty upset about this and thought I should have stayed with her. But then I was reminded that maybe that was not what Diana wanted. Maybe she wanted to be alone. So I think being in tune with my patients by putting my own desires and perceptions aside, will help me be more open to them*". (*C. Farrell, OMS III*, 2015)

This level of person-centered care and awareness relies on far more that than medical knowledge, it was amazing to us how much we learned through experience in 48 hours. The *Hospice Home Immersion* project obliged us to move out of our comfort zone; we each spent 48 continuous hours engaging with upwards of 14 dying patients and their families, we got little sleep, and when either of us did sleep we were in a bed where others have died before. This combination of experiences along with staff guidance accelerated our introspection and created comprehensive learning about end of life care. We gained competence throughout the 48 hour immersion as well as confidence.

4. Discussion

The topics of death and dying, palliative and end of life care are generally lacking in modern medical education. Most medical students will have little to no experience with these topics until they are faced with patients during their clinical rotations, for which they may receive varying levels of guidance from their superiors [7].

The *Hospice Home Immersion* project proved to be an effective learning tool for these students during their pre-clinical years. By living at the Gosnell Memorial Hospice Home for 48 hours, the students were able to develop skills on site and immediately utilize these with patients and families facing death. As evidenced through their ethnographic journaling, the students identified three themes that underscored their immersion: person-centered experiences, spectrum of communication, and introspection.

Through this project, the students were exposed to aspects of death and dying that were not easily explained pathologically. The students observed that not all symptoms experienced by the patients were physiologically based. This lead them to an understanding that medical care is multifactorial, and includes the physical, emotional, and spiritual aspect of a person. While this philosophy may be mentioned in traditional medical education practices, the emphasis has always remained in the physical dimension. By living in the hospice home, the students came to understand and appreciate the other aspects of a patient's life and how those aspects play a role in the dying process.

Communication is vital to healthcare. Reflected in their journals was the original belief that communication lies strictly in the verbal sphere. Often emphasized in medical education is the proper terminology and language to be used when communicating with patients, as well as with other providers. However, the non-verbal forms of communication that are often overlooked are essential when providing patient and family care. According to Mehrabian [14], the power of body language and voice tone in aiding communication is often not recognized

and yet it accounts for 93% of communication (voice tone accounts for 38%, and body language accounts for 55%), with words accounting for only 7% of communications [14]. Both students remarked that they felt trepidation about finding the correct words to use in the presence of dying patients. Once in the home they learned that other forms of communication are as meaningful and powerful, such as touch, silence, and mere presence. Care was effective without long conversations. Furthermore, the students developed the skills and aptitude to understand when a conversation may be warranted, and when other forms of communication would be best utilized.

Lastly, the *Hospice Home Immersion* project allowed the students to gain insight into their own attitudes and biases about death and dying. The process of living in a hospice home revealed students' attitudes about various disease processes, their personal experiences with death and dying, and their assumptions about how patients approach death. The project revealed the students' own biases on numerous occasions. Both students remarked on how their perceptions were misguided, be it in the form of how a patient wanted to die, or their perception of a family situation and disease. Overall, the *Hospice Home Immersion* project compelled the students to explore and challenge their assumptions surrounding the dying process. At the conclusion of their immersion, both students expressed gratitude for the experience, a greater depth of understanding of palliative and end of life care, and a newfound self-awareness.

Limitations

This article presents the experiences and outcomes of two of the twenty-six students who have conducted this project and research thus far. The results are specific to these two students and therefore may not be applied to all medical students. However, through this immersion project, each pair of students learning about palliative, end of life, and inter-professional care is being advanced; even if only by two students at a time. The *Hospice Home Immersion* project aggregate data analysis is in progress, however, as true with qualitative outcomes the results stated by each person who conducts this research is significant for that person.

5. Conclusions

Early medical education succeeds in equipping students with a foundation in the pathophysiology of disease and the performance of foundational clinical skills. There is considerably less emphasis placed on palliative and end of life care. Although, as previously stated, it has been well documented that the attitudes and values of a physician impact the care of their patients, assessment of the maturation of the student physician is lacking.

The 48 Hour Hospice Home Immersion project addresses this deficit by providing in-depth and experiential learning that encourages self-reflection. These pioneer students expressed a desire to mature as student physicians, to learn about person-centered care, and to operationalize medical humanities within a health care environment. The Gosnell Memorial Hospice Home and its inter-professional team of staff and volunteers taught these UNECOM medical students far more than mere medical care at the end of life. Instead, these students learned skills that will serve them throughout their careers, such as awareness of the person/patient, communication, and reflection/introspection. There is no question that medical education can do a better job in educating our future physicians in the field of palliative medicine. Maybe someday we can count on our medical schools to teach basic level competence in the care of the dying person, but will this instill confidence? As physicians they will be expected to perform palliative and humanistic skills, hopefully beyond the basic level [2].

The recommendations based on the outcomes of this project thus far, are to immerse our pre-clinical and clinical medical students into an in-patient hospice environment and have them work side by side with inter-professional staff and directly with patients and families during the palliative and end of life care phases. If experience is the best teacher then an essential first step, to learn what exemplary care entails for dying persons and their family, may be an extended immersion within an exceptional end of life care environment.

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Factors Affecting Treatment Interruption among TB Patients in Lagos Nigeria: Is There Any Need for Treatment Supporters?

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Abstract

Background: This study assessed treatment interruption of tuberculosis (TB) patients managed by treatment supporters and health care workers and other predictors of treatment interruption. Methods: A descriptive cross-sectional study was conducted. Four hundred and seventy new smear positive TB patients above 14 years of age were consecutively recruited between October 1 and December 31 2012 from 34 (23 public and 11 private) directly observed treatment short course (DOTS) facilities that offered TB treatment and microscopy services. They were followed up till treatment was completed. Logistic regression was used to assess the predictors of treatment interruption. Results: A significantly higher proportion of smokers (58.6% vs 38.3%, p = 0.030), patients supervised by treatment supporters (44.4% vs 34.7%, p = 0.032), patients not counselled before initiation of treatment (55.6% vs 38.2%, p = 0.041), patients managed at private DOTS facilities (50% vs 36.3%, p = 0.010) and TB/HIV co-infected patients (54.2% vs 38.6%, p = 0.038) had treatment interruption. Predictors of treatment interruption were supervision by treatment supporters, smoking, lack of pre-treatment counselling and TB/HIV co-infection. Conclusion: A higher proportion of patients supervised by treatment supporters had treatment interruption than those supervised by health care workers. There may be a need to review the concept of treatment supervision by treatment supporters in Lagos state Nigeria.

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Keywords

Treatment Supporter, Treatment Interruption, Tuberculosis, DOTS, Nigeria

1. Introduction

The National tuberculosis and leprosy control programme (NTBLCP) commenced the implementation of the directly observed treatment short course (DOTS) in Nigeria over two decades ago [1]. However, despite the availability of DOTS facilities in all local government areas (districts) in the country, Nigeria is far from achieving the global targets. Tuberculosis (TB) burden in Nigeria is estimated to be 318 per 100,000 [2] and less than twenty percent of the estimated TB prevalence was notified in 2013 [3]. In addition, the World Health Organization (WHO) estimated that 2.9% (2.1% - 4.0%) and 14% (10% - 19%) of new and retreated TB cases respectively had multi-drug resistant tuberculosis (MDR-TB) [3].

Although there is efficacious chemotherapy for TB treatment, therapy requires greater than ninety percent compliance to facilitate treatment success and reduce the emergence of MDR-TB [4]-[6]. Treatment interruption is a major obstacle in TB control [7]. Reasons for treatment interruption are complex and include patients' characteristics and income, the socio-cultural context, chronic nature of TB and patients' relationship with health care workers [8] [9].

Different methods such as monitoring system, pill counts, fixed dose combinations and supervised therapy were used previously to ensure patients' adherence to TB treatment [8] [10] [11]. Under the DOTS programme, TB treatment was initially supervised daily by health care workers during the first two months of treatment. However for many TB patients in most TB high burden countries including Nigeria, treatment interruption was a challenge because of financial accessibility to TB services as majority live some distance from the DOTS facilities and are often too weak to make frequent visits to access care [12].

To address this issue, "patient centered treatment" which allowed TB patients to determine whether treatment was supervised at the health facility by health care workers or at home by any treatment supporter of their choice was introduced [13]. The NTBLCP guidelines stipulated that treatment supporter can be a relation or someone (volunteer) close to the patient's home and has been trained to provide support for TB patients on treatment [14]. A study from Nigeria showed that few of the community volunteers served as treatment supporter and the community TB care in the country was not effective [15]. Studies from West and East Africa, Asia and South America have shown that the use of family members as treatment supporters was effective [16]-[20]. However, the effectiveness of treatment supporters depends on the type and age of treatment supporter, mode of selection, quality of training, proximity to patients, employment and educational status [21] [22].

Since the introduction of patient centered treatment in Nigeria in 2007, no study has assessed the role of treatment supporters in reducing treatment interruption among TB patients. This study assessed treatment interruption of patients managed by treatment supporters and health care workers and other factors associated with treatment interruption among TB patients managed in Lagos Nigeria.

2. Methods

2.1. Study Design

This study was a descriptive cross sectional study that assessed factors associated with treatment interruption of TB patients in Lagos Nigeria.

2.2. Study Background and TB Control in Lagos Nigeria

The study was done in Lagos state, the commercial nerve centre of Nigeria with an estimated population of 21 million [23]. TB control was coordinated by the Lagos State TB and leprosy control programme (LSTBLCP). The DOTS management of TB started in 2003 with the public sector and in 2008 the systematic engagement of the private sector began. TB diagnosis and treatment is free in the state. Duration of TB treatment was eight months consisting of two months intensive phase and six months continuation phase. Rifampicin, Isoniazid, Pyrazina-

mide and Ethambutol as fixed dose combination were given during the intensive phase while Ethambutol and Isonizid were given during the continuation phase as fixed dose combination. Usually patients were counselled on drug usage, possible side effects and consequences of treatment interruption before commencement of treatment. In order to reduce treatment interruption and loss to follow up, patients diagnosed of TB were managed at DOTS facility closest to their residents regardless of where TB diagnosis was made. Sputum microscopy, clinical assessment and drug intake were used to monitor TB treatment. Sputum microscopy was done at the 2nd, 5th and 7th month of treatment for smear positive patients, clinical assessment included weight monitoring and assessment of patient's records for regularity of drug intake [14].

All presumptive TB clients were offered HIV test at no cost. Determine (determine HIV-1/2 Alere Determine™, Japan 2012) and Uni-Gold™ (Trinity Biotech PLC, Wicklow, Ireland 2013) were used in parallel algorithm. STAT-PAK® was used as tie breaker for discordant results. TB/HIV co-infected patients were usually referred to anti retroviral therapy (ART) clinic for assessment and commencement of anti retroviral (ARV) drugs. In most cases TB drugs were commenced first before ARV drugs. TB/HIV co-infected patients were offered co-trimoxazole in addition to anti-TB drugs.

TB focal persons were in charge of each DOTS facility and records generated from each DOTS facility were sent to the local government TB supervisor who collates the records of DOTS facilities in the LGA (district) and send to the State TB coordinator who in turn forwards the state records to the national TB coordinator [14].

2.3. Population and Sample

Four hundred and seventy new smear positive TB patients were recruited from 34 DOTS facilities (23 from public and 11 private) out of the sample frame of 130 DOTS facilities provided by the Lagos State TB control officer between October 1 and December 31 2012. Selection criteria includes: provision of treatment and microscopy services and involvement in DOTS programme at least two years prior to data collection. Details of the sample size calculation were earlier published [24].

2.4. Study Procedure

A structured questionnaire was administered on all TB patients after recruitment into the study. Information on socio demographic data, clinical signs and symptoms and their duration, nature of the health facility first visited, whether treatment was supervised by treatment supporter or by health care worker at the DOTS facilities, whether counselling was done by health care worker before initiation of treatment were obtained. The weight, sputum and HIV test results were copied from patients' facility treatment card before commencement of patients on the 8 months anti TB regimen.

Drug use was directly observed daily during the intensive phase by the health care workers at the selected DOTS facilities for patients supervised by them, but during the continuation phase; patients were given monthly appointment. For patients not supervised by the health care workers, drugs were given to their treatment supporters to cover for two weeks. Treatment supporters were expected to supervise and record patients' treatment at home on a card. Empty drug blisters and the card (where drug intake was recorded) were presented to the health care workers at DOTS facilities before drug refill. The health care workers updated patients' health facility records from the card presented by the treatment supporters and days when patients interrupted treatment were recorded. Pattern of drug intake of patients supervised by the health care workers were also documented. Patients were followed up till completion of treatment.

2.5. Definition of Outcome Variable

In this study any patient who failed to collect drugs for two consecutive days during the intensive phase or two weeks after the expected date during the continuation phase were regarded as having interrupted treatment according to the NTBLCP guidelines [14].

2.6. Data Analysis

The Statistical Package of Social Sciences (SPSS) IBM version 19 was used for data analysis. Percentages, mean and standard deviation of numerical variables were determined. Chi squared test was used to compare categorical variables. Binary logistic regression was used to assess the predictors of treatment interruption. All (independent)

variable that were significant ($p < 0.05$) on bivariate analysis were entered at once (enter method) at the beginning to assess their predictive ability. Omnibus test of model coefficients value less than 0.05 and the Hosmer-Lemeshow goodness of fit test value greater than 0.05 were used to assess if the model was good quality. Confidence interval was set at 95% for all statistical tests. Statistical test was considered significant if $p < 0.05$.

2.7. Ethical Approval

Ethical approval was obtained from the Health Research and Ethical Committee of the Lagos State University Teaching Hospital. Written informed consent was obtained from TB patients before recruitment into the study.

3. Results

Of the 470 smear positive patients recruited for the study, 358 (23.8%) and 112 (76.2%) were from the private and public DOTS facilities respectively. Mean age of patients was 33.0 ± 11.6. About 60% (280) were males. 254 (54.1%) had secondary school education, while 417 (88.7%) earned less than 125 USD monthly. About 12% (56) and 6% (29) drank alcohol and smoked cigarette respectively. TB/HIV patients constituted 10.2% of the patients recruited as shown in **Table 1**. **Figure 1** shows that 186 (39.6%) of the patients recruited interrupted treatment.

A significantly higher proportion of smokers (58.6% vs 38.3%, $p = 0.030$), patients supervised by treatment supporters (44.4% vs 34.7%, $p = 0.032$), patients not counselled before initiation of treatment (55.6% vs 38.2%, $p = 0.041$), patients managed at the private DOTS facilities (50% vs 36.3%, $p = 0.010$) and TB/HIV co-infected patients (54.2% vs 38.6%, $p = 0.038$) had treatment interruption than non-smokers, patients supervised by health

Table 1. Socio demographic characteristics of TB patients.

Variable		Frequency n = 470 (%)
Age group (years)	15 - 24	114 (24.3)
	25 - 34	170 (36.2)
	35 - 44	105 (22.3)
	≥45	81 (17.2)
	Mean ± SD	33.0 ± 11.6
Gender	Male	280 (59.6)
	Female	190 (40.4)
Educational status	No formal education	29 (6.1)
	Primary education	84 (17.9)
	Secondary	254 (54.1)
	Post secondary	103 (21.9)
Income	<50,000 naira	417 (88.7)
	≥50,000 naira	53 (11.3)
Drink Alcohol	Yes	56 (11.9)
	No	414 (88.1)
Smoke cigarette	Yes	29 (6.2)
	No	441 (93.8)
HIV status	Positive	48 (10.2)
	Negative	394 (83.8)
	Not done	28 (6.0)
Type of DOTS facility	Public	358 (76.2)
	Private	112 (23.8)

NB: [#] = Includes single, divorced, separated and widowed.

workers, patients counselled before treatment, patients managed at the public DOTS facilities and HIV negative patients respectively (**Table 2**).

Binary logistic regression was used to assess the ability of the explanatory variables such as cigarette smoking, who supervised treatment, pre-treatment counselling, type of DOTS facility where treatment was administered and HIV status to predict treatment interruption. The full model containing all predictors was statistically significant ×2 (6, N = 442) = 36.48, p < 0.001. The model as a whole explained between 23.2% (cox and snell R squared) and 34.5% (Nagalkerke R squared) of the variance in treatment interruption and correctly classified 78.7% of cases. Lack of pre-treatment counseling made the strongest unique contribution (OR 5.338, 95% CI 2.520 - 11.308) to explaining treatment interruption (dependent variable). Other predictors of treatment interrup-

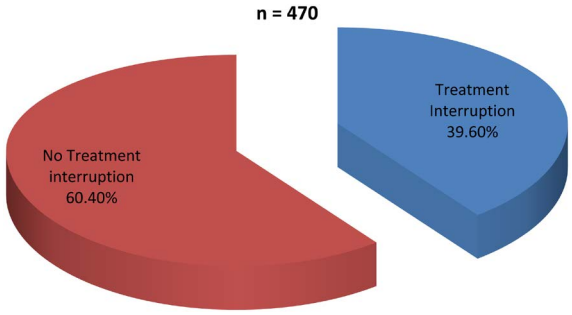

Figure 1. Prevalence of treatment interruption among TB patients.

Table 2. Factors associated with patient interruption among TB patients.

Variable		Patient interruption			
		Yes n = 284 (%)	**No n = 186 (%)**	**×2**	**p**
Age group (years)	<35	168 (59.2)	116 (40.8)	0.484	0.486
	≥35	116 (62.4)	70 (37.6)		
Gender	Male	168 (60.0)	112 (40.0)	0.052	0.819
	Female	116 (61.1)	74 (38.9)		
Educational status	Less than secondary	70 (61.9)	43 (38.1)	0.144	0.704
	Not below secondary	214 (59.9)	143 (40.1)		
Income	<125 USD	256 (61.4)	161 (38.6)	1.732	0.188
	≥125 USD	27 (51.90)	25 (48.1)		
Alcohol intake	Yes	36 (64.3)	20 (35.7)	0.396	0.529
	No	248 (59.9)	166 (40.1)		
Smoke cigarette	Yes	12 (41.4)	17 (58.6)	4.689	0.030
	No	272 (61.7)	169 (38.3)		
Treatment supervision	Treatment supporter	130 (55.6)	104 (44.4)	4.622	0.032
	Health care worker	154 (65.3)	82 (34.7)		
Pre treatment counselling	Done	268 (61.8)	166 (38.2)	4.164	0.041
	Not done	16 (44.4)	20 (55.6)		
Type of DOTS facility	Public	228 (63.7)	130 (36.3)	6.683	0.010
	Private	56 (50.0)	56 (50.0)		
HIV status	Positive	22 (45.8)	26 (54.2)	4.322	0.038
	Negative	242 (61.4)	152 (38.6)		
	Not done[#]	20 (71.4)	8 (28.6)		

NB: [#] = Not part of the analysis.

tion were smoking (OR 2.043, 95% CI 1.257 - 3.321), treatment supervision by treatment supporters (OR 2.043, 95% CI 1.257 - 3.321) and TB/HIV co-infected patients (OR 2.058, 95% CI 1.057 - 4.006) (**Table 3**).

4. Discussion

4.1. Prevalence of Treatment Interruption

In this study, about 40% of patients had TB treatment interruption, this is low compared with findings from Nigeria and elsewhere [25]-[27]. The reason for this finding is unknown. However, recruitment of patients from many DOTS facilities may be responsible for this finding. Many studies from Nigeria assessed the prevalence of TB treatment interruption in a DOTS treatment facility [25]-[27].

4.2. Factors Associated with Treatment Interruption

Adherence to treatment regimen is pivotal to TB control, and the involvement of the community is important in this regard especially where culturally appropriate support is available near a patient's home [28]. Treatment supporters have evolved to assist TB control programs in low-income countries and those with high TB prevalence [22]. Anecdotal evidence however shows that the quality of support provided by treatment supporters varies significantly [22]. In our study, patients managed by treatment supporters were twice likely to have treatment interruption than patients managed by health care workers. This is contrary to the findings from Tanzania and Ghana which reported better treatment outcome and lower treatment interruption among patients supervised by treatment supporters. However, the closer the treatment supporter live to the patient, the better the treatment adherence [16]-[18].

Although the NTBLCP guidelines stipulates that treatment supporters must live close to the patients and be trained by the health care worker [14], there was no mechanism in place to verify patients claim whenever a treatment supporter was presented. In addition, the health care workers often do not follow the NTBLCP recommended standard operating procedures for the engagement of treatment supporters. A potential challenge to the effectiveness of treatment supporters is quality control especially when observation is performed by a non-medical supporter outside a health facility [16] [22]. It has also been suggested that treatment supporters should not be seen as a "quick fix" for failing TB control programs. For better effectiveness of treatment supporters, there must be a well organized and functional health system [22].

Counselling for TB adherence is a process of improving patients' knowledge about TB, enhancing their belief to complete treatment and developing skills to overcome negative family and community environments [29]. It often strengthens patients to overcome problems that may jeopardize adherence [29]. Studies have shown that counselling enhances patients' adherence to TB treatment [30]-[32]. In this study, pre-treatment counselling was associated with treatment interruption. Patients who reported that they were not counselled before the commencement of TB treatment were five times likely to interrupt treatment than those who reported they were counselled before treatment. Similar finding was obtained in a randomized controlled intervention trial. Counselling improved patients' knowledge about TB and minimized treatment interruption [33], although earlier studies from Pakistan and elsewhere showed that counselling had no significant impact on treatment interruption even with monetary incentive to patients [29] [34]. Other authors have however suggested that the success of any counselling program is dependent not only on the qualification and competence of health care workers but also their attitudes towards patients, their desire to improve patients' treatment outcome, and the type of intervention [30] [31] [35] [36].

Table 3. Predictors of treatment interruption.

Variable	B	Wald	p	OR	95% CI
Smokers	0.963	4.771	0.029	2.620	1.104 - 6.218
Treatment supporter	0.714	8.304	0.004	2.043	1.257 - 3.321
No pre-treatment counselling	1.675	19.121	<0.001	5.338	2.520 - 11.308
TB/HIV co-infection	0.722	4.506	0.034	2.058	1.057 - 4.006
Treatment at private DOTS facility	0.405	2.111	0.146	1.500	0.868 - 2.590

Odds ratio adjusted.

Cigarette smoking has been shown to be associated with TB infection, disease and death [37]-[39]. It can also affect treatment outcomes and studies have shown that smoking was associated with unsuccessful treatment outcomes, default and relapse [40]-[42]. In this study, smokers had over 2 fold risk of treatment interruption than non-smokers. This may be because majority of smokers (96.6%) in this study were males and men have demonstrated poor adherence to TB treatment [43]. This may be because of economic reasons because traditionally, men are expected to provide for the family and time taken out for medical visit may mean less income. In addition they may need to take permission from their work place which may not be possible with paid employment [44].

Patient with TB/HIV co-infection in addition to TB care need to get HIV care and may interrupt TB treatment more frequently than those who are HIV negative. This may be due to the cost of attending two clinics especially if both clinics are not located within the same hospital [45]. In this study TB/HIV co-infected patients were twice likely to interrupt treatment than TB/HIV negative patients. Several studies have reported factors associated with treatment interruption of TB/HIV co-infected patients. Believe in efficacy of TB treatment, pill burden, side effects of drugs, failure of health workers to counsel patients of the potential side effects, HIV related stigma and discrimination and lack of disclosure were reported in studies from Nigeria, Ethiopia and Nepal as factors affecting adherence in TB/HIV co-infected patients [45]-[51].

4.3. Limitation of the Study

No enquiry was made in this study to ascertain whether treatment supporters actually lived close to TB patients, truly observed the patients' treatment or personally filled the treatment supporters' card. This study assessed factors associated with treatment interruption under programmatic condition.

5. Conclusion

A higher proportion of patients supervised by treatment supporters had treatment interruption than those supervised by health care workers. There is a need for health care workers at DOTS facilities to adhere strictly to the guidelines for selection of treatment supporters and take time to counsel TB patients before they are commenced on treatment. The need for further research to assess the effectiveness of treatment supporters in Lagos State Nigeria cannot be over emphasized.

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Competing Interests

Authors have declared that no competing interests exist.

Authors Contribution

AAO conceived the study, involved with data collection, data analysis and discussion. OJD wrote the methodology and was involved in the writing process, AFO was involved in drafting the manuscript and revision. ENA and HAA were involved in literature search and proof reading the manuscript.

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Scientific Research Publishing

Coronary Embolization and Myocardial Microinfarction: MR Imaging and Histopathologic Characterization

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Abstract

Magnetic resonance imaging (MRI) has been proven to reliably assess regional perfusion and left ventricular (LV) function of microembolized myocardium. The visibility of microinfarct on delayed enhancement MRI (DE-MRI) is limited and dependent on technical and biological issues. Furthermore, MRI underestimates total microinfarct size compared with microscopy. MRI studies revealed that the presence of microemboli in pre-existing acute infarct delays infarct healing and magnifies LV remodeling. Discrimination of acute from chronic microinfarct is based on presence of inflammatory cells, edema and scar tissue, respectively. These noninvasive findings highlight the importance of prognostic utility of MRI and warrant larger clinical studies or registries to evaluate the significance of presence of focal microinfarct. Serial microscopic studies revealed that intravascular microemboli migrate into the extravascular space and this migration process is a function of time. This phenomenon may limit the use of microemboli therapy in occluding hemorrhagic blood vessels or treating tumors. Despite current standard of care, existing methods and therapies do not prevent coronary embolization nor reverse their deleterious effects.

Keywords

Percutaneous Coronary Revascularization, Coronary Microembolization, Myocardial Microinfarct, Magnetic Resonance Imaging, Microscopy

1. Introduction

Every minute more than one person suffers from acute myocardial infarction (AMI) in the United States [1].

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Thromboembolic occlusions occur as a result of spontaneous clot formation and dislodgment of atherosclerotic plaques [2]. For patients presenting with an acute myocardial infarction, numerous revascularization strategies have been developed in an attempt to reduce infarct size, limit onset of heart failure and improve both left ventricular (LV) function and clinical outcome. Previous studies showed that over one million percutaneous coronary intervention (PCI) procedures are performed annually in the United States [3]. Paradoxically, revascularization of infarct-related coronary artery could produce further damage to previously undamaged myocardium by dislodged microemboli and a phenomenon called reperfusion injury. Clinical studies confirmed that PCI to break down large clots generates microemboli that occlude downstream microvessels [3] [4]. Cuculi *et al*. showed that 5% - 30% of the patients suffered from coronary embolization after PCI [5] and the effects on myocardium varied from non-symptomatic to sudden death [6]-[8]. This review addresses the use of MRI and microscopy in characterizing coronary microembolization and myocardial microinfarct.

2. Coronary Emboli

Autopsy studies showed that coronary embolization occurred 3 to 4 times more often in the left coronary artery than in the right, and in the left anterior descending (LAD) than in the left circumflex coronary artery [9]. The middle range of the size of coronary micoemboli retrieved after coronary intervention was 47 - 2503 μm. Coronary embolization describes a process where aggregated dislodged platelets, atherothrombotic debris and released vasoactive substances induce microvascular obstruction, inflammation and patchy microinfarction. Coronary embolization also occurs in spontaneous atherosclerosis plaque rupture, valvular disease, endocarditis, arrhythmias, heart-lung bypass surgery, congenital heart disease, hypertension, diabetes, systemic lupus erythematosus and sickle cell disease.

In a recent study, Grutzendler *et al*. [10] proved that microemboli could be cleared from microvessels by angiophagy, in which emboli are were engulfed by the endothelium and they translocated through the micro-vascular wall. The engulfment of emboli by the endothelial membrane projections leads to reestablishment of blood flow, vessel sparing and salvaged ischemic tissues. The molecular control of the extravasation mechanism involves mechanotransduction, vascular plasticity, cytoskeletal dynamics and remodeling of endothelial junctions [11].

3. Cardiac Injury Biomarkers

Cardiac injury occurs when there is disruption of myocyte membrane integrity that results in the loss into the interstitial and blood of intracellular constituents, such as creatine kinase MB (CK-MB), troponin I, myoglobin, heart-type fatty acid binding protein, and lactate dehydrogenase.CK-MB and troponin elevations in the blood are indicative of myocardial injury in patients. Thus, the diagnosis of AMI has become increasingly dependent on serum CK-MB and troponin I. However, the elevation of these two biomarkers after microinfarction can be undetected due to their dilution in the large sinus blood flow. After PCI cardiac injury biomarkers have been used to document early myocardial injury caused by microemboli [12] [13]. A large meta-analysis of 23,230 patients with stable or unstable angina undergoing PCI with follow-up for 6 - 34 months compared with the data from healthy volunteers showed a close relationship between CK-MB concentration and mortality rate, even at a minor increase of CK-MB 1 - 3 × conferring a relative risk of death of 1.5 (95% CI 1.2 to 1.8) [14].

In an experimental study [15], investigators found after 24 hrs PCI that plasma concentrations of CK-MB and troponin I were significantly higher after delivery of 16 mm^3 and 32 mm^3 microemboli to the LAD coronary artery compared with baseline. However, there was no significant difference at this time point in troponin I after 16 mm^3 (0.52 ± 0.28 ng/ml) and 32 mm^3 (0.68 ± 0.4 ng/ml) or CK-MB (1670 ± 370 U/L and 1060 ± 235 U/L, respectively). After 72 hrs, however, the concentration of troponin I was significantly higher (1.34 ± 0.43 ng/ml, $P < 0.05$) in animals that received 32 mm^3 than the ones receiving 16 mm^3 volume (0.55 ± 0.28 ng/ml), suggesting that the method of assay of cardiac injury biomarkers at early stage is not sensitive and specific enough to differentiate the effects of varied microemboli volumes.

4. Non-Invasive Imaging

Ischemic heart disease can be detected directly on positron emission tomography (PET) and single-photon emission computed tomography (SPECT), MRI, computed tomography (CT), and indirectly on electrocardiography

(ECG), cardiac injury biomarkers, ventriculography, and echocardiography. PET, SPECT, CT and echocardiography have been the clinical modalities for assessing myocardial perfusion and viability, while intravascular imaging methods, such as optical coherence tomography (OCT) and intravascular ultrasound (IVUS), characterize plaque composition (large necrotic cores, high plaque volume, thin-capped fibroatheroma) [16]-[18]. Echocardiography is the most commonly used clinical method for quantification of global and regional LV function in patients with ischemic heart disease [19]. Investigators also used two-dimensional (2D) speckle-tracking images to measure regional strain and strain rate [20]. This method enables quantifications of LV remodeling [21] and LV reverse remodeling [22].

Porto *et al*. [23]-[25] were the first to count coronary microemboli in real-time during PCI using high intensity transient signals (HITS) derived from Doppler guidewire. Coronary microembolization has also been linked to myocardial contractile dysfunction, malignant arrhythmias, perfusion deficits and coronary reserve impairment [7] [23] [24] [26]. Few clinical studies showed the potential of MRI in simultaneously estimating LV function and regional perfusion as well as visualization of acute and scarinfarct. However, visualization and quantification of acute and scar microinfarct are still clinically challenging. The variability of patient populations, microemboli volume/size, territory of feeding vessels and cardiac motion play roles in drawing sound conclusions on the deleterious effects of microemboli in addition to the poor spatial resolution of current diagnostic scanners and the lack of suitable quantitative techniques. Investigators found that the controlled preclinical animal models were suitable for resolving the above complexities of myocardial ischemia, reperfusion injury and coronary microembolization. Large animal models were used in many studies to recapitulate the clinical scenario of percutaneous coronary intervention [15] [27]-[30]. In these studies, coronary angiography showed normal epicardial and side branches arteries after embolization. The sizes of chosen microemboli in our studies where in the middle range of the size of microemboli escape the distal protective devices during PCI and the number of microemboli was based on previous animal studies [15] [27]-[30]. It should be noted that currently there is no animal model of spontaneous plaque rupture resulting in coronary microembolization.

5. Magnetic Resonance Imaging

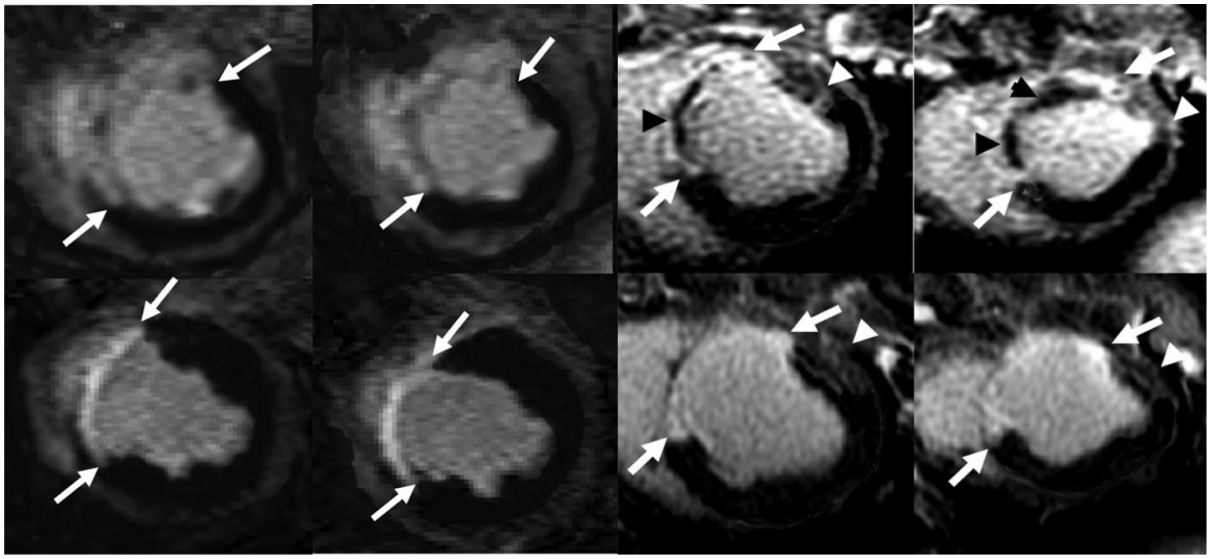
Conventional MRI sequences have historically been used in characterization of cardiac anatomy and follow-up of congenital heart disease. Large scale studies and case reports have reported the clinical utility of MRI for diagnosis of acute and scar infarcts.

MRI is evolving at a rapid pace. Among numerous interesting developments in the sensitivity and diagnostic accuracy of MRI, many can be expected to be directly useful for the evaluation of ischemic heart disease. As hardware and coil technology are improving, image quality and diagnostic yield will be more consistent for small and focal areas of myocardial microinfarction, inflammation and fibrosis. The new MRI sequences provide early recognition of cardiac disease, and important new insights into efficacy of novel therapeutics that are currently in trails. For example, Kwong *et al*. [31] reported that even small infarct (1.4% of LV mass) identified on DE-MRI portended a > sevenfold increased risk for major adverse cardiac events. Bodi *et al*. [22] found that assessment of infarct and microvascular obstruction on MRI soon after ST-segment elevation myocardial infarct (STEMI) enabled the prediction of reverse remodeling.

6. Myocardial Viability

Inversion recovery techniques, such as gradient echo and modified Look-Locker (MOLLI), are used to visualize, quantify and map myocardial infarct (MI), microvascular obstruction (MVO) and peri-infarct zone, while dynamic contrast enhanced MRI with blood pool contrast media is used to estimate blood volume and characterize vascular permeability. The most commonly used DE-MRI sequence for myocardial viability is 2D inversion-recovery gradient echo sequence (2D IR-GRE) [32]. DE-MR images are acquired 8 - 10 min after injection of (0.1 - 0.2 mmol/kg) extracellular gadolinium-chelates (**Figure 1**). The LV mass and extent of microinfarct are quantified using semiautomatic threshold methods [15] [29] [33] [34]. The recently developed delayed contrast enhanced three-dimensional (3D) IR-GRE has been validated against 2D IR-GRE in patients with AMI [35]-[37] and in swine hearts subjected to microinfarction. The modalities provided similar estimation of large infract and microinfarct [38].

A previous DE-MRI clinical study demonstrated a large variation in the size of microinfarct (0.7 - 12.2 g or 0.4% - 6.0% LV) [12]. In an experimental DE-MRI study, investigators found a positive correlation between



(a) (b)

Figure 1. (a) DE-MR images obtained from an animal subjected to 90 min LAD occlusion/revascularization show hyperenhanced contiguous AMI (top row, white arrows) with small MVO zones at 3 days post intervention and hyperenhanced, thin scar infarct at 5 weeks (bottom row, white arrows) with compensatory hypertrophy in remote myocardium. (b) The images obtained from an animal subjected to 90 min LAD occlusion/microembolization/revascularization show hyperenhanced contiguous AMI (top row, white arrows), hypoenhanced large MVO in the core (black arrowheads) and patchy microinfarct in the border zone (white arrowheads) also 3 days post intervention. At 5 weeks, larger scar infarct and compensatory hypertrophy was evident (bottom row, white arrows) in the 90 min LAD occlusion/microembolization/revascularization animal compared with 90 min LAD occlusion/revascularization animal.

microemboli volume and microinfarct size 3 days after embolization [33]. From a clinical perspective, patchy microinfarct in the peri-infarct zone is of pivotal importance for the prognosis and recovery of LV function and arrhythmia [38] [39]. An electrophysiological study implicated the heterogeneity of depolarization and dispersion of repolarization to microinfarct within the peri-infarct zone. Investigators found that microinfarct in the peri-infarct zone is the key substrate in arrhythmia-related sudden cardiac death [40].

In a swine model, delivery of 66 mm^3 microemboli also caused arrhythmia and 25% mortality rate within the first 24 hrs [41] and this mortality rate is comparable to that observed in 90 min LAD occlusion/revascularization animals [42]. Experimental DE-MRI study demonstrated the patchy myocardial enhancement 6 hrs after microspheres injection [33] [43] [44]. Breuckmann *et al*. [43] indicated that a threshold of 5% was necessary for visualization of microinfarct on DE-MRI. Furthermore, investigators reported that only visualized microinfarct on DE-MRI cause LV dysfunction in patients [45] (**Figure 2**). Our MRI study showed that both visible and invisible microinfarct resulted in LV dysfunction [34] because this imaging modality underestimates microinfarct size compared to microscopy.

In a recent proof-of-concept study, we demonstrated that mildly injured myocardium subjected to 40 min LAD occlusion then microembolization did not manifest greater susceptibility to infarction compared with solely embolized non-ischemic myocardium and 40 min LAD occlusion groups [15] (**Figure 3**).

The area at risk corresponds to the perfusion bed of embolized artery and myocardial salvage index is area at risk minus microinfarct divided by area at risk. Breuckmann *et al*. [43] reported that T2-weighted imaging was limited in detecting edematous area at risk 8 hrs after embolization. They attributed this limitation to the small difference (10%) in water content between embolized and remote myocardium [30]. More recently, T2-MRI for measurement of area at risk, based on the formation of interstitial edema, has been seriously criticized and disputed [46]. In a recent publication in patients and animals, Kim *et al*. [47] indicated that T2-weighted MRI did not measure salvageable myocardium, but true infarct.

The equilibrium state of distribution of MRI contrast media in myocardium can be used for evaluating myocardial viability based on the measurement of extracellular volumes. The distribution of MRI contrast media in normal myocardium is in the intravascular and interstitial spaces, while in infarcted myocardium, the myocyteslose their

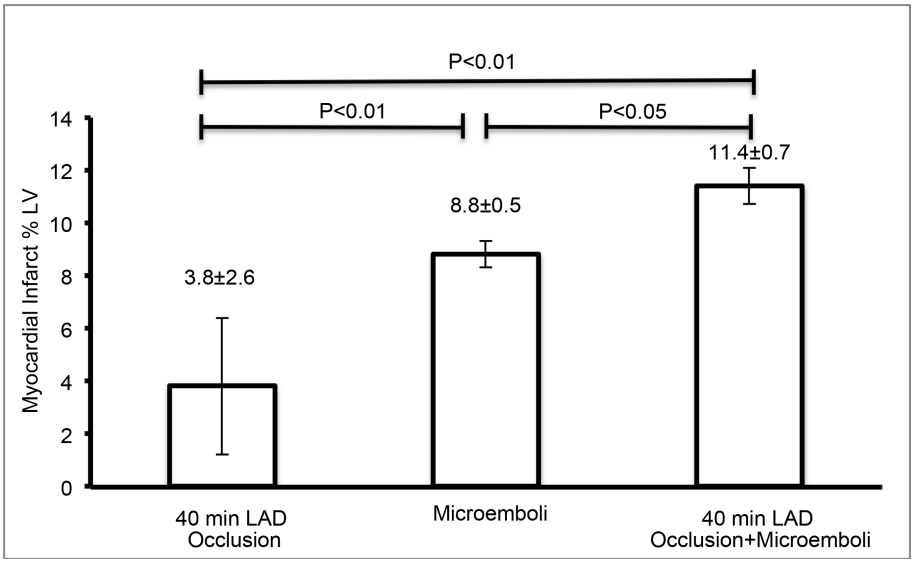
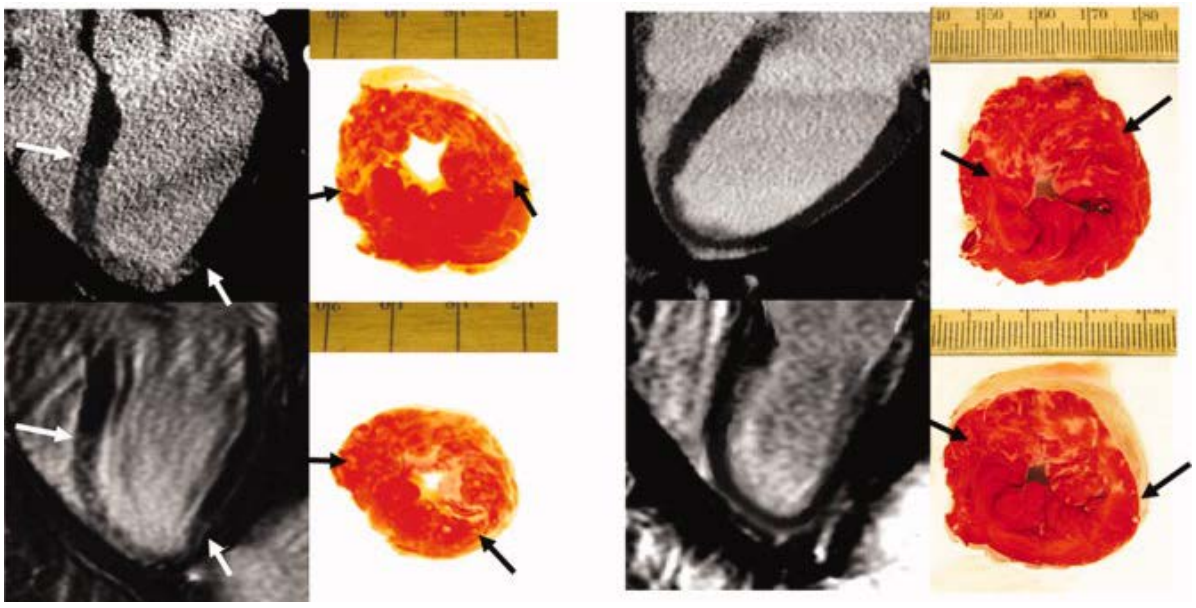


Figure 2. Bars show the incremental increase in myocardial infarct sizes in animals subjected to 40 min LAD occlusion/revascularization, coronary microembolization by 32 mm^3 microemboli or the combination of both insults.



(a) (b)

Figure 3. The variability in visualizing myocardial microinfarct on long-axis DE-MDCT (top) and DE-MR (bottom) images in two animals after administration of the same volume/sizes of microemboli (16 mm^3 of 40 - 120 μm). DE-MDCT and DE-MR long-axis view images show speckled microinfarct along the inter-septal LV wall ((a) white arrows), but not (b) side images. Unlike MDCT and MRI, TTC-stained sections show microinfarct in both animals (black arrows).

cellular integrity to become a part of the extracellular space, thus providing larger distribution volume compared with normal myocardium. The technique was first used in detecting small myocardial damage in rats subjected to 20 min LAD coronary artery occlusion/revascularization. Investigators found clear difference in fractional distribution volume between normal myocardium (18%) and patchy infarcted myocardium (32%). Ischemic myocardium demonstrated dispersed focal cellular necrosis involving 18% of the cells per field under microscope [48].

The recent advancements of high field MR scanners and sequences for mapping T1 relaxation time allow scientists and clinicians to explore minor pathologic changes in myocardium. Investigators showed that T1

mapping sequences had the potential to demonstrate regional T1 changes associated with edema and diffused fibrosis [49]-[55]. T1- and T2-mapping images showed increases in native T1- and T2-relaxation times and a decrease in T1-relaxation time in MI post-contrast media injection [55] [56]. Native T1-mapping drew more attentions of clinicians, as it did not require contrast media and was accessible in the context of renal impairment or contrast allergies [56] [57].

7. Multi-Detector Computed Tomography

With the improvements in spatial and temporal resolution sand reduction in radiation exposure, MDCT has evolved into major clinical noninvasive coronary artery imaging modality. MDCT has been used for visualizing microinfarct and detecting LV dysfunction in embolized myocardium in beating swine heart model [41] [58]-[60].

Iodinated contrast media for MDCT have extracellular distribution, thus theoretically, their kinetics is parallels to those of gadolinium chelates. On the basis of this hypothesis Jablonowski *et al*. [60] assessed myocardial extracellular volumes in normal myocardium, contiguous infarct and patchy microinfarct. They found that the fractional distribution volume was 24% in viable myocardium, 36% in microinfarct after delivery of 16 mm^3 microemboli, 41% in microinfarct after delivery of 32 mm^3, 55% in large infarct after 90 min LAD occlusion/revascularization and 56% after 90 min LAD occlusion/revascularization with delivery of 32-mm^3 microemboli. The microscopic measurements confirmed MDCT data. Regression analysis revealed excellent correlation between regional myocardial extracellular volume on MDCT and microscopy (r^2 = 0.92). On micro-CT, Malyar *et al*. identified *in vitro* the patchy pattern of perfusion in microembolized myocardium and attributed it to a random distribution and clustering of microemboli in microvessels [61]. However, computed tomography has limitations, such as poor temporal resolution, artifacts, radiation exposure and contrast-induced nephropathy.

8. LV Function

Accurate assessment of LV function is essential for the diagnosis, therapeutic management and prognosis. LV remodeling is characterized by alterations in myocytes and extracellular matrix resulting in alteration geometry and architecture of the chamber. Deformation pattern based on radial, circumferential and longitudinal myocardial strains have been used to determine the severity of myocardial dysfunction. Two-dimensional echocardiography with Doppler is the common method for assessing regional and global function of the LV. However, echocardiographic measurements present with several limitations, mainly with regard to through-plane motion and poor acoustic windows.

MRI offers great advantage over echocardiography by providing a set of contiguous short-axis MRI LV and RV slices from the base to the apex, and long-axis views. The ECG-triggered cine MRI sequences provide data on cardiac mass, volumes, and 3-D strains (radial, circumferential and longitudinal) after microembolization [29] [33] [62]-[64]. Such data can be combined with myocardial perfusion, viability and coronary flow.

Carlsson *et al*. [62] demonstrated the deleterious effects of relatively large microemboli (100 - 300 μm diameter, 70 mm^3) on regional LV radial strain in swine model. Cine MRI showed the changes in LV volumes and ejection fraction at 1 hour and 1 week after microembolization compared to baseline, which might be attributed to the persistent decline in the radial strain of the embolized region (**Figure 4**). In another study, a decline in ejection fraction from 49% ± 1% at baseline to 29% ± 1% at 1 hr (P = 0.02) and 36% ± 1% at 1 week after delivery of 7500 microemboli of 100 - 300 μm diameter was documented [63]. There was no correlation between the ejection fraction and microinfarct size (r = 0.20) at 1 hr or at 1 week (r = 0.54). Similarly, there was no correlation between the ejection fraction and the extent of perfusion deficit at 1 hr (r = 0.27) or 1 week (r = 0.39) (P = not significant for all). Our findings of persistent declines in regional and global functions at 1 week using relatively large emboli (100 - 300 μm in diameter) are in line with findings in sheep using 90 μm emboli [65]. However, heterogeneity among studies exists. For example, a study in dogs showed that LV dysfunction occurred within hours after delivering of a small embolic agent (42 μm) followed by a complete recovery of function within 5 - 6 days [27]. The investigators suggested that the decrease in function resulted from myocardial inflammation mediated by TNF-α.

We also compare the effects of two microemboli volumes (16 and 32 mm^3) on regional and global function. We also found that the effect of 32 mm^3 microemboli on radial stain was broader (involved at least 4 segments

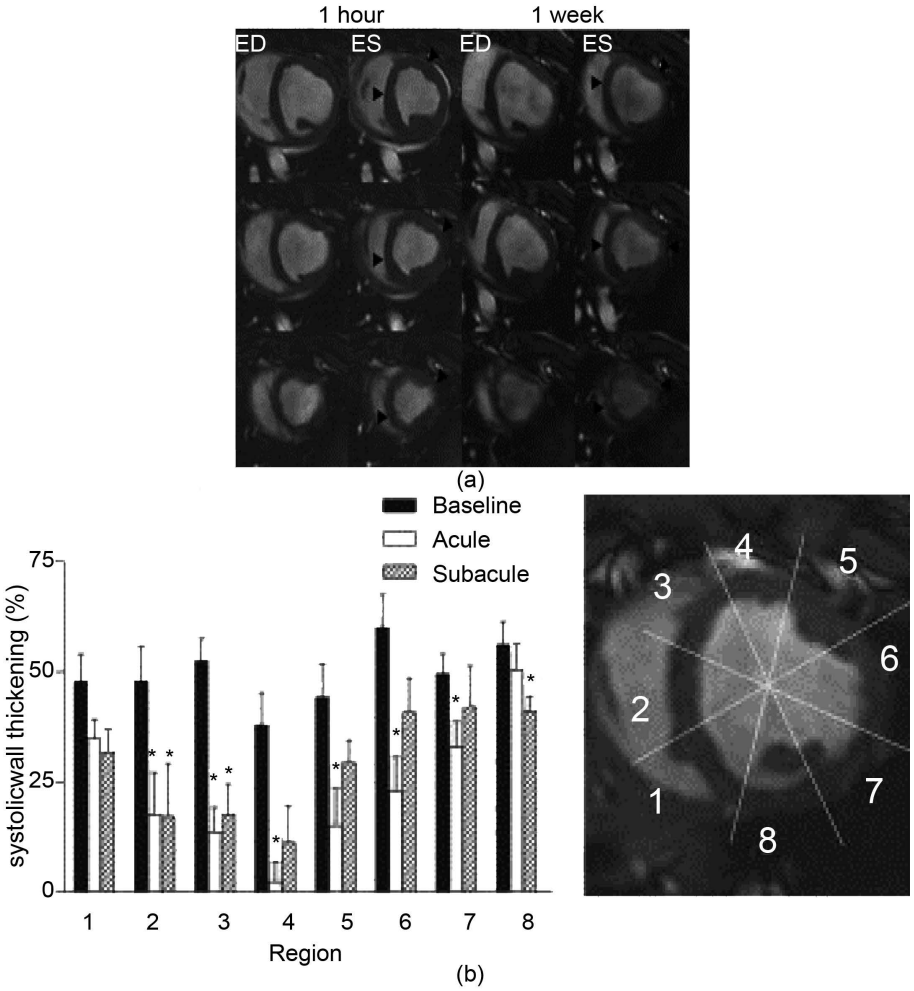


Figure 4. (a) Three slices cine MRI at 1 h and 1 week after microembolization acquired at end diastole and end systole. Decreased function is seen in the anteroseptal wall (black arrowheads). (b) Radial strain (systolic wall thickening) is shown in eight segments at baseline, 1 h, and 1 week. The area of microembolization is located between segments 2 and 5 and shows dysfunction. The MR image (b) shows the location of the regions used for analysis of wall thickening. $^{*}P < 0.05$ compared to baseline.

in basal, mid and apical MRI slices) than animals that received 16 mm^3 microemboli. At the global level, microemboli caused acute increases in LV diastolic and systolic volumes (**Figure 5**). Furthermore, LV ejection fraction was significantly lower in animals that received 32 mm^3 than 16 mm^3 microemboli [34]. Serial MRI studies showed that coronary microembolization led to LV remodeling and persistent decline in systolic wall thickening [41] [63] [66] [67].

We also explored the potential of cine MRI for quantifying the acute (3 days) effects of defined microemboli volumes and sizes on LV function in preexisting AMI in a swine model [34]. Animals subjected to LAD occlusion/microembolization/revascularization showed greater LV wall thinning, decrease in ejection fraction and increase in end-systolic volume than controls and animals subjected to LAD occlusion/revascularization (**Figure 6**). Quantitative analysis showed a total of 576 segments with systolic wall thickening were graded as normal, with a thickening of more than 30% (192, 112, and 64 segments in control, LAD occlusion/revascularization, and microemboli in preexisting AMI groups, respectively); hypokinetic, with 10% - 29% thickening (48 and 48 segments in LAD occlusion/revascularization, and microemboli in preexisting AMI groups, respectively); akinetic, 0% - 9% (32 and 32 segments, respectively); and dyskinetic, −10% to 0% (0 and 48 segments, respectively). Dyskinesis and paradoxical systolic-wall thinning were observed only in LAD occlusion/microembolization/revascularization group. Circumferential and longitudinal wall strains were also depressed after coronary

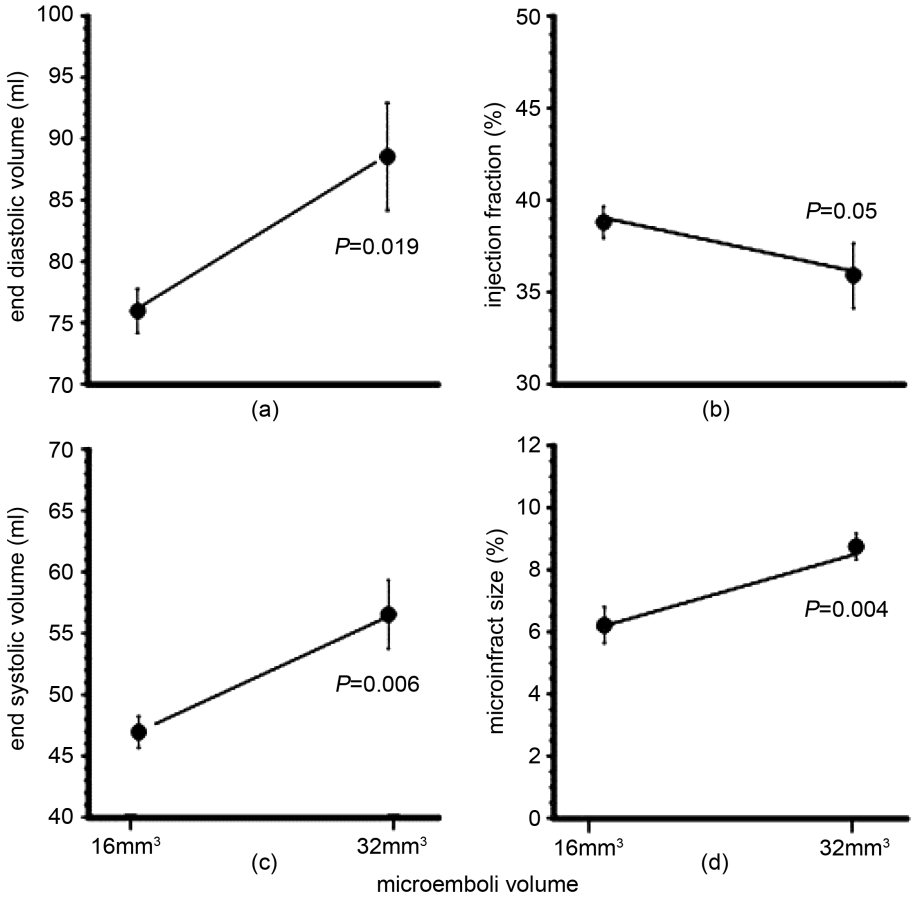
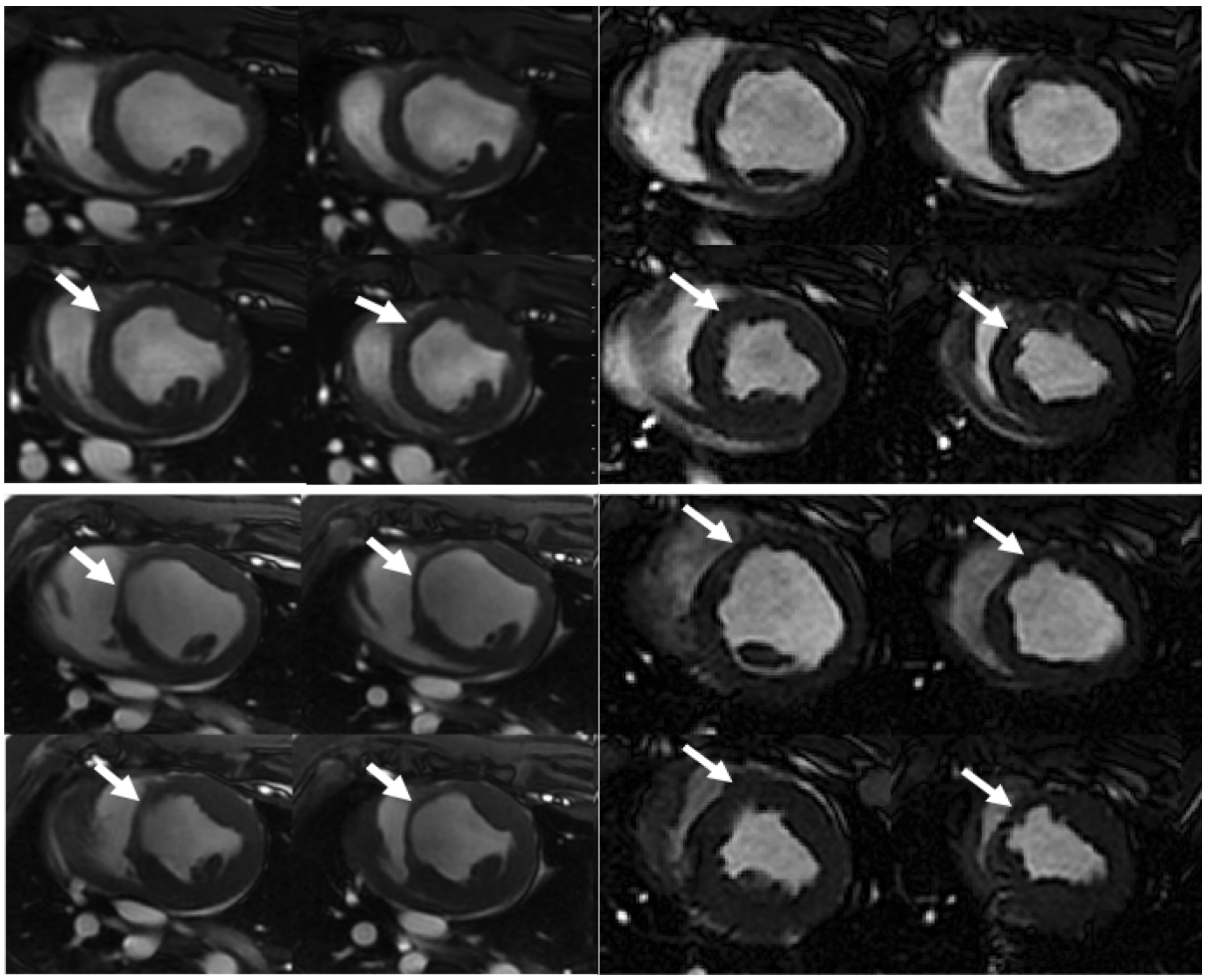


Figure 5. The effects of two different microemboli volumes are shown on LV end diastolic volume (a), end systolic volume (c), ejection fraction (b) on cine MRI and speckled enhanced microinfarct size (d) on DE-MRI. All the parameters show significant difference related to the microemboli volumes.

microembolization [64]. Suhail *et al*. [64] found that cine and tagged MRI sequences were useful for measuring left/right ventricles longitudinal and circumferential strains in patchy microinfarct and large infarct, respectively. HARP and plane metric software are used to quantify circumferential and longitudinal strains in microembolized infarct. Investigators observed that coronary microemboli caused greater impairment in LV circumferential strain and dyssynchrony than 90 min LAD occlusion/revascularization animals despite the significant differences in infarct sizes. Microemboli also caused a significant decrease in peak systolic strain rate of remote myocardium and LV dyssynchrony. Compensatory increase in longitudinal strain of RV free wall was also observed in response to microemboli delivered in the LAD and LAD occlusion/revascularization animals. This study concluded that 1) coronary microemboli with or without AMI core caused complex myocardial injury and ventricular dysfunction that were not replicable in solely AMI and 2) there was a disproportion in the declines of circumferential strain, dyssynchrony, and infarct size of animals subjected to microembolization and AMI. Clinical study showed that longitudinal strains measured on cine MRI correlated well with infarct sizes [68], while Galiuto [69] indicated that the improvement in longitudinal strain was an index of myocardial viability, associated with global LV improvement and possibly reverse remodeling, which is an important predictor of a favorable long-term outcome.

9. Myocardial Perfusion

The severity and extent of myocardial ischemia is a key to decision-making for revascularization. With commencing myocardial ischemia, a cascade of cellular, functional and electrocardiographic events ensues. Thallium-201 scintigraphy studies demonstrated that coronary stenos is causes perfusion deficits [70] [71]. Investigators also observed a mismatch between LV dysfunction and epicardial coronary blood flow after revascularization



(a) (b)

Figure 6. Cine MRI. (a) Multislice diastolic (top row) and systolic (bottom row) cine MR images acquired at 3 days (top block) and 5 weeks (bottom block) from an animal subjected to 90 min LAD occlusion/revascularization. White arrows point to the site of infarction and show wall thinning at 5 weeks. (b) Multislice diastolic (top row) and systolic (bottom row) cine MR images acquired at 3 days (top block) and 5 weeks (bottom block) in an animal subjected to 90 min LAD occlusion/microembolization/revascularization. At 5 weeks, the site of infarction (arrows) show greater wall thinning compared with 90 min LAD occlusion/revascularization.

[72]. First pass MRI also detected myocardial perfusion deficits in patients after PCI [24] [73].

In general, microembolized myocardium with and without pre-existing infarct is defined as hypoenhanced zone on first pass MRI. Unlike DE-MRI, perfusion imaging has the potential to detect early effect of microemboli (as early as 1 hr) on myocardium perfusion (**Figure 7**). Maximum upslope, maximum SI and time to the peak obtained from first pass MRI perfusion are the best indices to estimate regional perfusion deficits [33] [34] [62] [74]. Quantitative analysis of perfusion parameters revealed in these studies that the maximum signal intensity and time to peak were lower and longer, respectively, in both acute and scar microinfarct compared with remote myocardium. Selvanayagam *et al*. [75] used first pass perfusion and DE-MRI to demonstrate perfusion deficits and new microinfarct 24 hrs after PCI. Choi *et al*. [45] found an association between perfusion deficits and discrete AMI in patients after PCI.

In an experimental study, Mohlenkamp *et al*. [76] investigated the changes in coronary microcirculation (intramyocardial microvascular blood volume, perfusion, transit time and pattern of microvascular injury) in response to different sizes of microemboli. They observed that 100 μm microspheres resulted in patchy plugging, while 10 μm microspheres induced contiguous hemorrhagic myocardial injury. Skyschally *et al*. [73] demonstrateda lack of changes in baseline coronary blood flow after stepwise repeated injections of microsphere (42

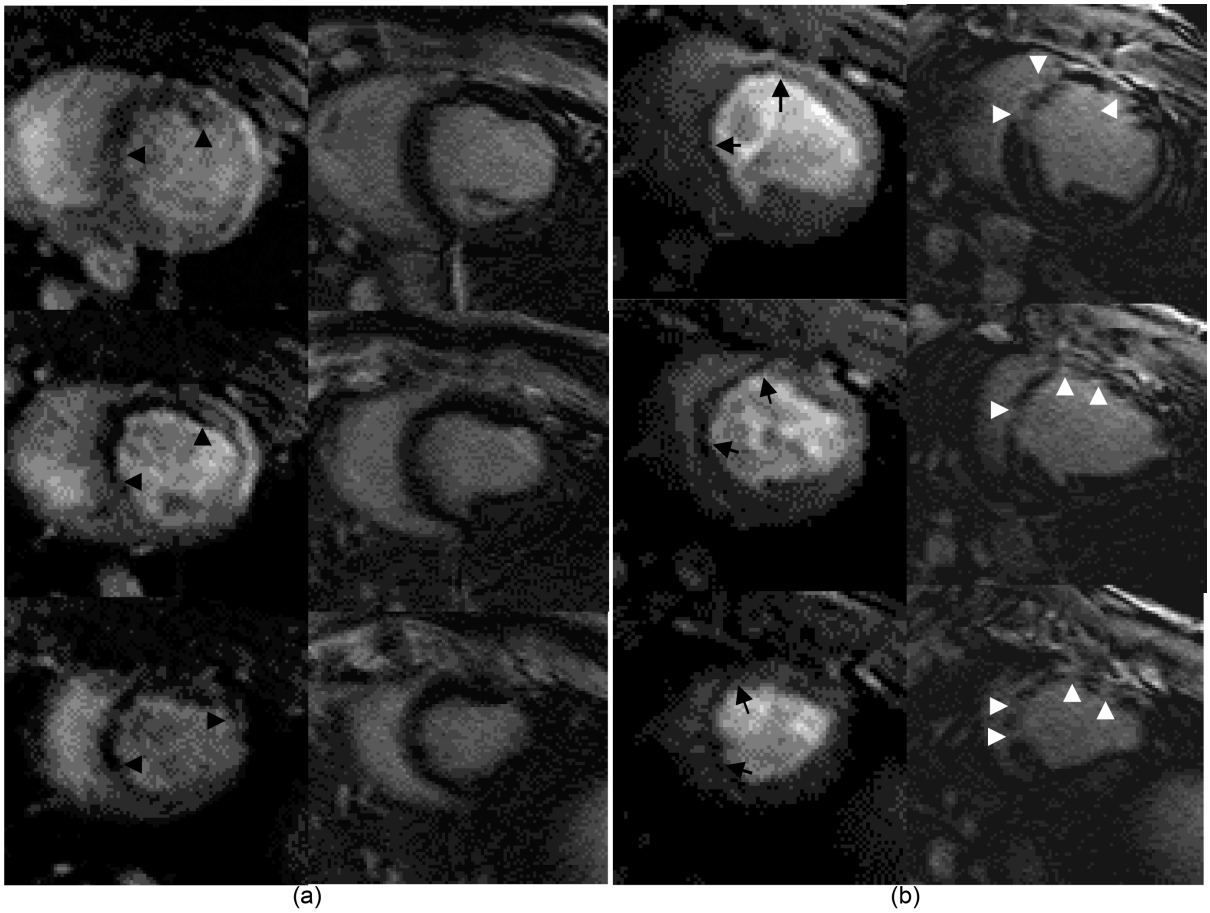


Figure 7. Multislice first pass perfusion DE-MR images acquired 1 hr ((a), left block) and 1 week ((b), right block) after LAD embolization show the persistent perfusion deficit in embolized myocardium. DE-MRI images acquired 1 hr after embolization failed to demonstrate myocardial microinfarct. On the contrary, DE-MRI provided evidence of microinfarct at 1 week.

μm diameter) using Doppler flowmeter. In contrast, Ma *et al*. observed in swine the reductions in coronary flow reserve and LV ejection fraction 6 hours after emboli injection (42 μm, 120,000) into LAD. The animals showed complete recoveries of flow reserve and LV ejection fraction, but LV dilation, 1 week later [66]. Bai *et al*. [77] also reported a persistent LV dysfunction and progressive remodeling in swine model 28 days after repeated microsphere injection.

10. Histopathology

Gu *et al*. [8] observed under light and electron microscope that delivery of automicrothrombotic particles into the coronary arteries induced microthrombosis, damage of vascular endothelium, and microinfarct. Other investigators showed that monocytes/macrophages dominated the cellular infiltrates for the first 2 weeks after MI and participated in wound healing [78] [79]. Frangogiannis [80] classified the healing process of myocardial infarct into 3 distinct but overlapping phases: the inflammatory phase, the proliferative phase, and the maturation phase, while Nahrendorf *et al*. [81] summarized the roles of monocytes/macrophages in infarct healing, including 1) release inflammatory mediators; 2) release proteases; 3) phagocytose apoptotic and necrotic myocytes and neutrophils and other debris; 4) promote angiogenesis; 5) transport reparative enzymes and prosurvival factors; and 6) stimulate collagen synthesis and deposition by myofibroblasts. It has been shown that multiple injections of microspheres also induce myocardial injuries similar to those seen in patients after revascularization [77]. **Figure 8** shows the patchy microinfarct and infiltration of inflammatory cells in infarcted myocardium 3 days after microembolization. Dead myocytes were generally found in clusters (large or small). Furthermore, microinfarcts

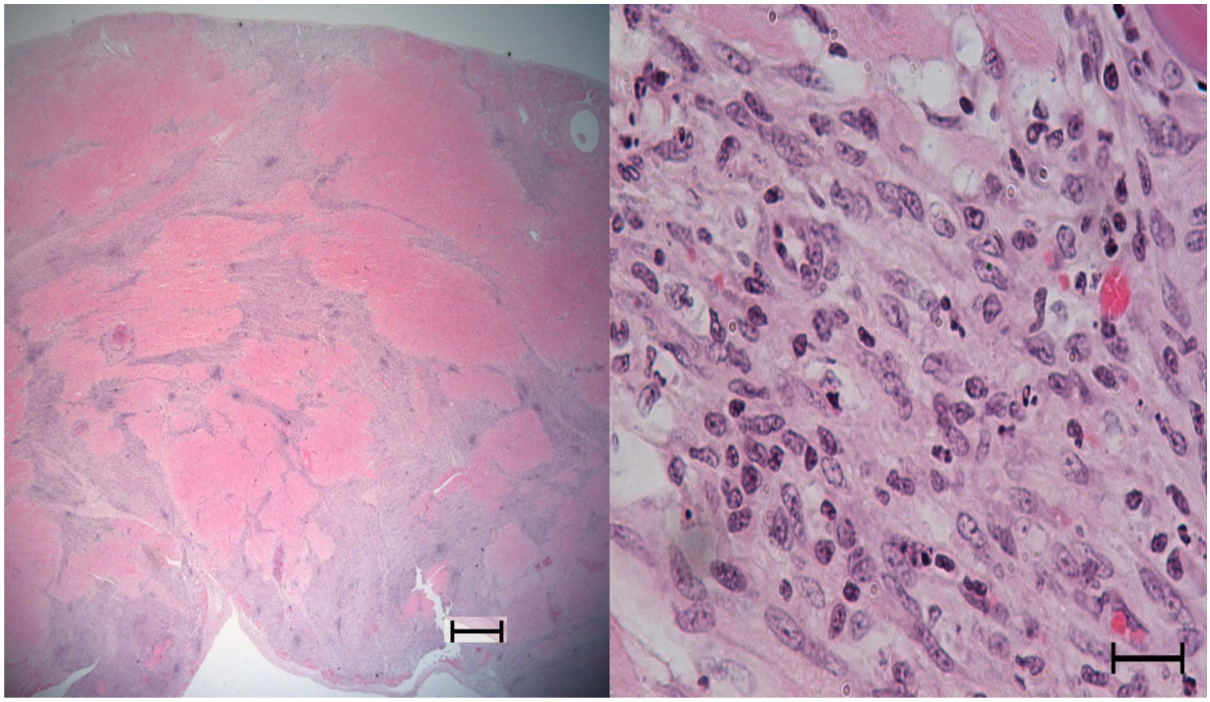


Figure 8. Microscopic cardiac sections stained with hematoxylin-eosin show the patchy microinfarc and infiltration of inflammatory cells in infarcted myocardium 3 days after coronary microembolization (10×, scale = 500 μm and 100×, scale = 50 μm).

showed severe coagulation and a contraction band of necrosis with complete loss of cellular and vascular architectures. The islands of microinfarct spread randomly within the LAD territory and were relatively small and less dense in animals that received small volume of microemboli (16 mm^3) compared with animals that received large volume (32 mm^3). Three days after delivery of solely emboli, necrotic myocytes were replaced with aggregates of mononuclear cells (mostly macrophages) [34]. Bai *et al*. [77] observed an early increased inflammatory activity followed by persistent pro-inflammatory cytokines protein expression and collagen deposition. The use of iron particles, as MR contrast medium, might be helpful in noninvasively identifying and quantifying temporal changes in myocardial inflammation [82]. Monitoring macrophages/monocytes infiltration might be useful for predicting clinical outcomes and treatment efficacy.

Gu *et al*. [8] observed under light and electron microscopy that delivery of automicrothrombotic particles into the coronary arteries induced microthrombosis, damage of endothelial cells and myocardial microinfarct. Others found in the first week that intravascular microemboli are surrounded by fibrin, aggregated platelets and leukocytes [58]. Scar microinfarct were microscopically differentiated from acute microinfarct by the lack of infiltration of inflammatory cells and fibrosis [15] [29] [83]. At 5 weeks, reperfused infarct without microemboli showed remodeled blood vessels with a thick wall and small lumen, but no evidence of obstructed vessels, while revascularized infarct with microemboli showed almost complete obstruction of microvessels (**Figure 9**). Furthermore, at this time intravascular microemboli started to cross the vascular wall and by 8 weeks they were settled in the interstitial space. Microscopy also disclosed the presence of single, multiple of various sizes of microemboli outside of the intravascular compartment (peri-vascular space) (**Figure 10**).

It has been shown that slow infarct healing can lead to LV remodeling, infarct rupture and death [84]. Interstitial edema was evident at 5 weeks in remote myocardium of animals subjected to LAD occlusion/microembolization/revascularization, but not in LAD occlusion/revascularization animals (**Figure 11**). Furthermore, these animals showed less infarct resorption and LV dilation at 5 weeks in animals subjected to LAD occlusion/microembolization/revascularization compared with LAD occlusion/revascularization animals (**Figure 1**). Thus, our experimental study [29] proved the conjecture by Kloner [85] and Wu [86] that MVO delayed/inhibited optimal infarct healing by slowing the delivery of inflammatory cells and nutrients. Infarct resorption (shrinkage) is related to the deposition of compact fibroblasts that have smaller sizes than cardiomyocytes. A clinical MRI

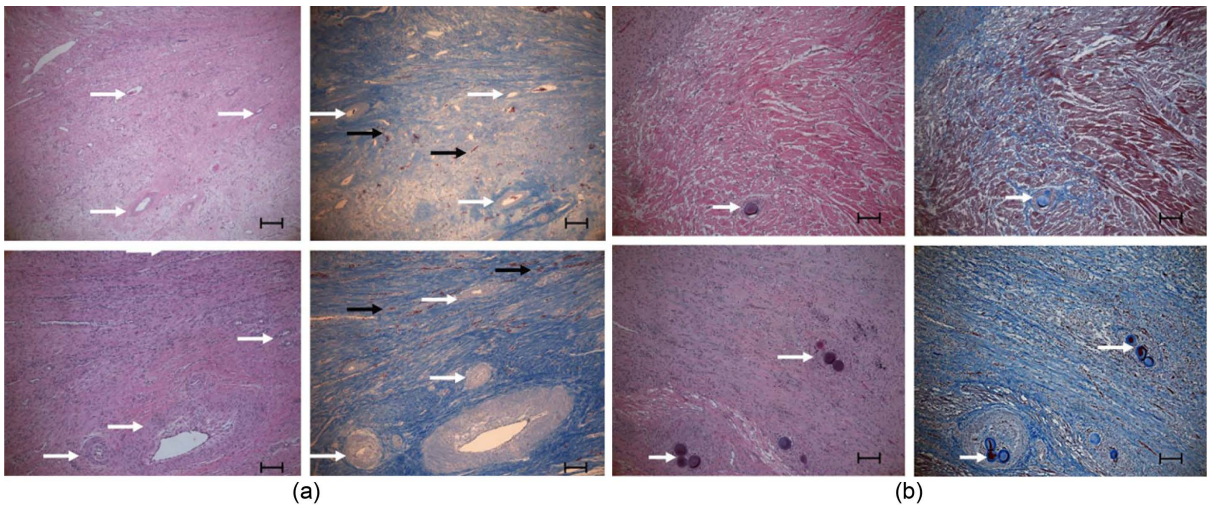


Figure 9. Microscopic cardiac sections stained with hematoxylin-eosin and Masson trichrome stains show the differences at the cellular and vascular levels between 90 min LAD occlusion/revascularization at 5 weeks (a) and 90 min LAD occlusion/microembolization/revascularization at 5 weeks (b) animals. The former animal showed remodeled blood vessels with a thick wall and small lumen (white arrows) and new patent vessels (black arrows), but no evidence of obstructed vessels. The latter animal showed complete obstruction of microvessels by fibrotic tissue, debris and inflammatory cells (10×, scale = 500 μm) [15] [29] [83].

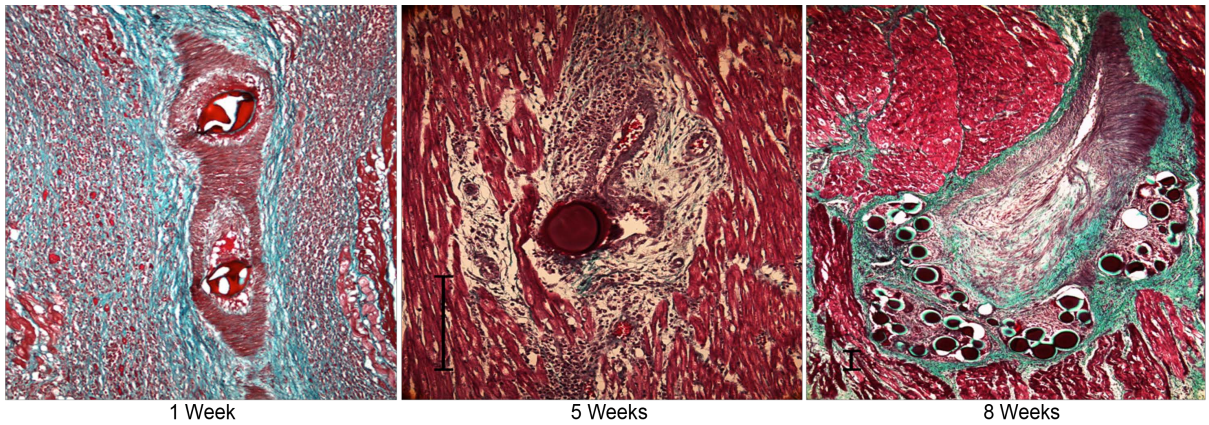
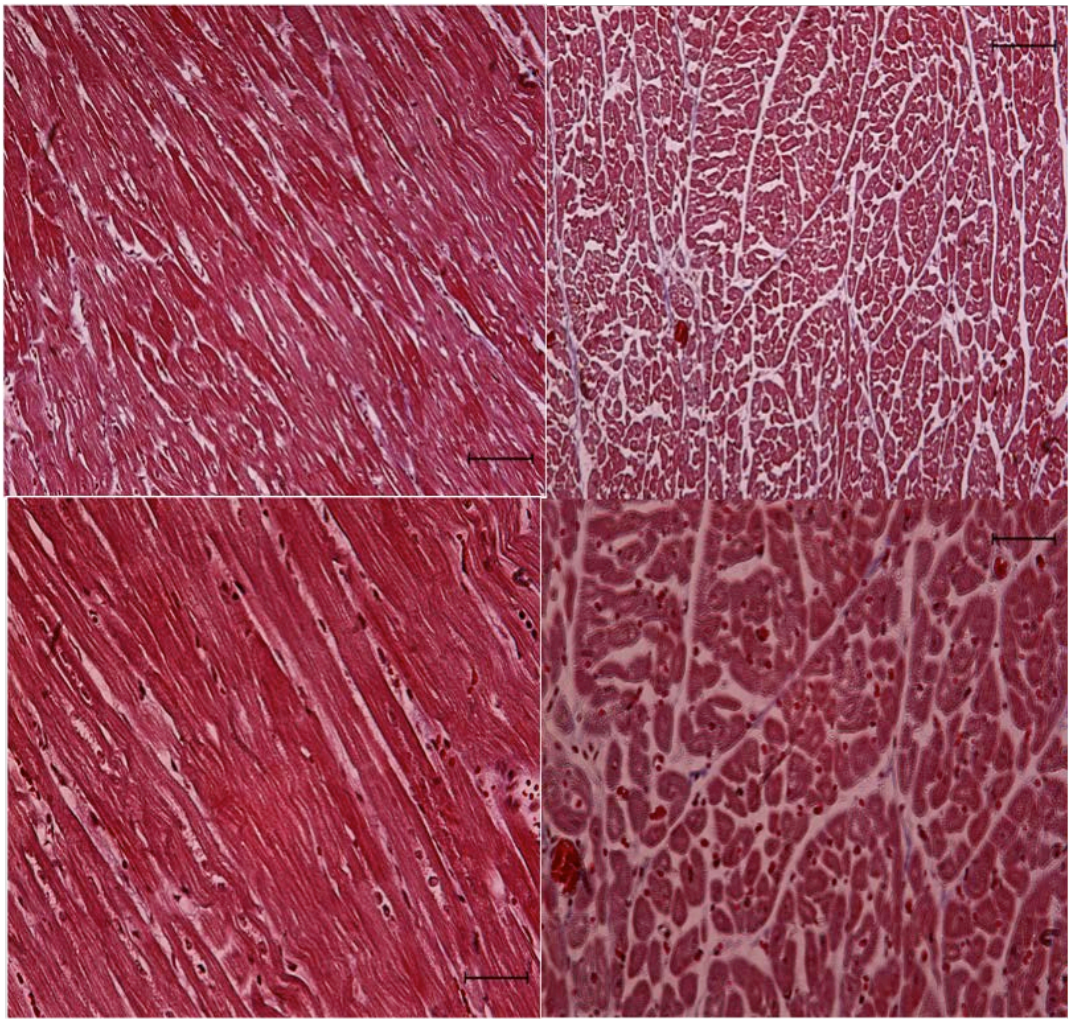


Figure 10. Migration of intravascular coronary microemboli into the interstitial space as a function of time. At 5 weeks the microemboli start to migrate into interstitial space and 8 weeks almost all microemboli are located in the interstitial space. (top ×40, middle and bottom ×200 [15] [83]).

study showed that the resorption of large infarct was faster than small infarct [87]. On the contrary in swine, MRI and histologic study demonstrated that the resorption of microinfarct was faster than large infarct [15]. The resorption of solely microinfarct and large infarct at 5 weeks were 60% and 25%, respectively, [15] [88]. Choi *et al*. [89] and Inkangisorn *et al*. [90] found in patients a decline in infarct sizes of 27% and 31%, respectively, 2 months after infarction. These results indicate that the percent resorption of myocardial infarct is identical in humans and swine.

At the global level, LV mass was the greatest in animals subjected to occlusion/microembolization/revascularization compared to control and 90min LAD occlusion/revascularization animals, which is partially related to the interstitial edema (**Figure 12**). Additionally, scar microinfarct on DE-MRI (1.3 ± 0.9 g) and histochemical staining (1.9 ± 1.2 g) are smaller than on microscopy (3.3 ± 0.5 g, $P < 0.05$) [15] [83]. The difference in the measurements was even greater in animals subjected to LAD occlusion/microembolization/revascularization compared with animals subjected to only microembolization (9.0 ± 0.6 g on DE-MRI, 10.7 ± 1.9 g on histochemical staining and 15.6 ± 2.8 g on microscopy, $P < 0.05$).

Investigators observed apoptotic bodies in microembolized and remote myocardium using cleaved caspase 3



(a) (b)

Figure 11. Microscopic sections show remote myocardium in animals subjected to 90 min LAD occlusion/revascularization (a) and 90 min LAD occlusion/microembolization/revascularization (b). The interstitial edema is evident only in LAD occlusion/microembolization/revascularization 5 weeks after the interventions (×40 and ×100).

stain [33] [91]. Reactive oxygen species have been shown to exert direct inhibitory effects on myocardial function in vivo and to have a critical role in the pathogenesis of myocardial stunning [92]. Activation of lectin-like oxidized low-density lipoprotein receptor 1-dependent mitochondrial pathway, caspase-8-dependent pathway, [93] and tumor necrosis factor α (TNF-α) [94] induced apoptosis. TNF-α is a cytokine released during myocardial infarction. It's expression during the healing phase was not confined to the infarct or peri-infarct zone and could be localized in the viable remote myocardium [33] [94], in which remodeling is ongoing [29].

11. Treatment of Myocardial Microinfarct

Chen *et al*. [95] recently found that glucocorticoid therapy improved LV function after coronary embolization through the suppression of transforming growth factor-beta 1 (TGF-β1)/Smad 3 and connective tissue growth factor. It also attenuated LV remodeling caused by microinfarct [67]. Jin *et al*. [96] found a less decline in regional wall motion in the embolized area in animals treated with glucocorticoid (methylprednisone, as anti-inflammatory therapy) than control animals at 6 hrs after coronary microembolization. Methylprednisone administration ameliorated myocardial dysfunction (88.6% ± 7.6%) compared with control group (47.7% ± 4.7%; $P < 0.001$) at 6 hrs after embolization. The systolic wall-thickening index was at the baseline, 96.3% ± 8.2%. The LV ejection fraction decreased from 49.9% ± 3.5% at baseline to 34.6% ± 3.7% at 6 hours ($P < 0.001$) in the

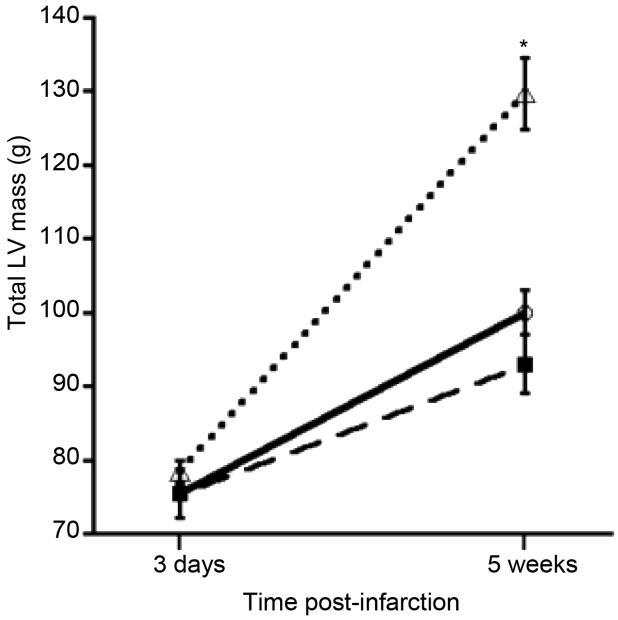


Figure 12. A plot showing the differential increase in LV masses over the course of 5 weeks in control (dashed line), 90 min LAD occlusion/revascularization (continuous line) and 90 min LAD occlusion/microembolization/revascularization animals (dotted line). $^{*}P < 0.001$ compared with the other two groups.

control group, which was significantly less in treated group from 47.1% ± 3.8% to 42.5% ± 3.9%. Other found that statins, antiplatelet agents, and coronary vasodilators can protect AMI from microemboli and additional microinfarct when administered prior to PCI. Distal protection devices can retrieve atherothrombotic debris, but their effects are controversial.

Experimental studies revealed that the phosphatase and tensin homolog deleted on chromosome ten (PTEN) were proteins regulating inflammation and apoptosis. Apoptotic bodies were evident in microembolized and remote myocardium using cleaved caspase 3 stain [33] [91]. This protein is highly expressed in reperfused AMI and it enhances inflammation after embolization [97]. Investigators found that inhibition of PTEN improved myocardial function by attenuating myocardial apoptosis [28].

Additional observations in microembolized myocardium are: calcium deposits were also evident in scar microinfarct [33] and intravascular microemboli were present in the perivascular space 5 - 8 weeks after delivery of microemboli, suggesting migration of microemboli across vascular wall. This phenomenon may limit the use of microemboli therapy in treating tumors or occluding hemorrhagic blood vessels. Recent studies showed that emboli were cleared from the microvasculature by either hemodynamic pressure or angiophagy, in which emboli were engulfed by the endothelium and translocated through the microvascular wall [10]. Furthermore, it has been recently proposed that monitoring of coronary microvascular function might facilitate tracking injectable macromolecular nanoparticles that use the leaky vasculature after MI for infarct healing [98].

In summary, existing diagnostic algorithms for the determination of the potential etiologic substrate of coronary microembolization and myocardial microinfarction processes are largely based on the identification of myocardial ischemia/injury and inflammation. This review shows an incremental diagnostic utility of MRI in coronary microembolization and myocardial microinfarction beyond the algorithm established by current guidelines. It also adds to the existing body of information on the utility of MRI to identify potentially reversible myocardial abnormalities and to define the prognostic use of MRI for the treatment of injured myocardium. MRI has been proven to reliably assess regional perfusion and LV function of microembolized myocardium. The visibility of microinfarct on DE-MRI is limited and dependent on technical issues, such as optimization of the inversion time, elimination of motion artifacts and MR contrast media relaxation time *in situ* and their kinetics (wash-in/wash-out) as well as biological issues, such as microemboli volume, age of microinfarct and collateral circulation. Cardiac MRI should focus in the future on improving the spatial resolution and acquisition time to overcome some of these limitations. Additionally, for advancement of clinical care it is of paramount importance to develop innovative techniques for preventing coronary microemboli formation and treating micropin-

farct. Microscopic examination of biopsy is a gold standard method for confirming the presence of microemboli in coronary microvessels, but biopsy is not recommended after PCI in routine clinic.

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Conflicts of Interest

None.

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Rationalizing Optimal Timing for Adjuvant Hormone Therapy for Patients with Breast Cancer: Impact on Limited Resource Countries

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Abstract

Modern day cancer chemotherapy is complex and involves multiple drugs given either sequentially or concurrently, as an adjuvant or neo-adjuvant. Besides the concentration of the drug, timing, duration and sequencing of individual drugs in combination with other similar agents play a vital role in the final therapeutic outcome. This study constitutes an exhaustive overview of current knowledge of timing and sequencing, specifically of Tamoxifen, based on tumor's hormone receptor status, as part of a comprehensive treatment plan. It has become apparent that inappropriate timing or sequencing can be detrimental. On the other hand, appropriate timing and sequencing of Tamoxifen, based on breast cancer cell-biology, pharmacokinetics and pharmacodynamics of drugs, the body's homeostatic response to drugs; surgery and radiation, yield huge benefit for locoregional control, long-term survival and reducing complications in patients with breast cancer. *Conclusion*: A rational plan for use of Tamoxifen has been recommended, based on this study; for optimal therapeutic benefit. It has also been suggested that in receptor "unknown cases", it is beneficial to prescribe Tamoxifen, since 75% of breast cancers are likely to be estrogen receptor positive and side effects can be minimized with planned vigilance.

Keywords

Optimization, Tamoxifen, Breast Cancer, Limited Resource Countries

1. Introduction

Tamoxifen (estrogen receptor modulator) and Letrozole (an aromatase inhibitor) are used extensively for ER

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and PR receptor positive, for both pre- and post-menopausal patients, with breast cancer. In an adjuvant setting, when Tamoxifen is used as part of treatment, the optimum timing of sequencing hormones with chemotherapy, radiotherapy and surgery has been frequently queried.

On the clinic floor, the appropriate time to start and stop hormones if prescribed and the ability to stop at any time, if at all, or until the regimen finishes, are often asked by both patients and nursing staff alike.

The majority of cancer treatments involve multiple chemotherapy agents and multiple treatment modalities. Due to variable interaction with hormones and other therapeutic agents, it is important to have a clear understanding of the optimum dosage, sequencing and timing of each agent and each modality. This is likely to have a profound impact on the final outcome.

Optimizing time and sequence of surgery, radiotherapy and chemotherapy in the management of breast cancer can improve therapeutic benefits [1]. The addition of hormones and anti-hormones to breast cancer treatment armamentarium has improved the total outcome significantly [2] [3].

Sequencing chemotherapy with radiation and surgery for patients with breast cancer has been well studied in classical investigative work by Abraham Recht [1] Adjuvant chemotherapy, adjuvant radiotherapy, preoperative chemotherapy/radiotherapy, radiotherapy sandwiched between cycles of chemotherapy, and neo-adjuvant chemotherapy all have been extensively studied by many investigators following Recht's historical publication.

Studies to establish optimal sequencing of hormones with chemotherapy, radiotherapy and surgery appear to have generated moderate interest for clinical trials, in spite of hormones' vital role in the management of breast cancer. There have been several experimental studies [4] [5] confirming that it is worth noting that the role of Tamoxifen and estrogen on cell cycle and cell cycle dependent agents used for breast cancer may impact adversely or advantageously.

In 1981, it was proposed that adjuvant hormone therapy should be deferred during the course of chemotherapy. The simple assumption that Tamoxifen, being an estrogen receptor blocking agent, was likely to interfere with the dynamics of cycling cells, delay progression from G0 to G1 and increase in the proportion of G0/G1 phase, at the same time reducing the proportion of "S" phase. This dual effect reduces effectiveness of cell cycle dependent chemotherapy [6]. In fact, adjuvant estrogen might enhance the cell cycle and increase the proportion of "S" phase, making chemotherapy more effective [7].

The proposal at that point failed to raise intellectual curiosity. Recent rediscovery of the original proposal delighted the authors that the hypothesis is alive, being accepted (through the persistence of other investigators) and is practiced universally.

This study attempts to rationalize the importance of optimal sequencing of hormones, *i.e.*, Tamoxifen, etc., with other primary treatment modalities for breast cancer.

2. Role of Estrogen on Cell Cycle Regulation and Tumor Growth

Estrogen increases the proportion of "S" and "G2-M" phases in cycling estrogen receptor positive and negative breast cancer cell lines. The effect of platelet derived factors that initiate cell cycles crossing over from one phase to the next is influenced by estrogen. The epidermal growth factor and insulin-like growth factor stimulate cycling cells to progress from G0/G1 phase to "S" phase, which is also influenced by estrogen [8]. Thus, estrogen itself, by promoting cell cycle "S" phase accumulation, promotes tumor growth, invasion and metastasis.

3. Effect of Tamoxifen on the Cell Cycle

Tamoxifen, by contrast, is an estrogen-blocking agent. It exerts its anti-tumor activity by competing for and binding to cytoplasmic estrogen receptor proteins in the tumor. The drug acts as an estrogen antagonist [9]. Thus it promotes accumulation of G0/G1 phases of cycling breast cancer cells and impairs crossing over and progression forward to "S" phase. Tamoxifen retards the cell cycle by impairing the effect of growth factors on cells and reducing the effect of cell cycle dependent anti-cancer agents, *i.e.*, cytotoxic drugs and ionizing radiation. However, Tamoxifen is also known to arrest G2/M phase [10]. G2/M phase is sensitive to radiation and certain microtubule-disrupting chemotherapy agents, *i.e.*, Vincristine, Taxol, etc.

4. Cycling Cell Dependent Chemotherapy Agents

Cytotoxic cancer chemotherapeutic agents are essentially "cell cycle dependent" for their oncotoxic activity, ir-

respective of cell cycle phase dependence or independence. Chemotherapy works by killing actively growing and dividing cells. There are more dividing and metabolically active cells in cancerous tissue than its normal counterpart, which is responsible for the drug's therapeutic advantage.

Both genetic and epigenetic mechanisms are engaged in transformation of normal cells to cancer cells, affecting the orderly expression of cell cycle regulatory proteins. Transformed malignant cells have deregulated CDK activity, offering the malignant cells the advantages of faster cell cycle and growth [11]. Hence, cycling cells in "S" and "M" phases are important for chemotherapy to be effective as opposed to resting phase of G0 and G1 phase.

Any condition or agents that accumulate cells in G0 phase or arrests progression from G0 to G1 to "S" phase would inhibit the effectiveness of cancer chemotherapy. Tamoxifen does just that. Tamoxifen, by retaining more cells in G0/G1 and G2 phases of the cell cycle, induces relative resistance to the majority of cancer chemotherapy agents, compromising their therapeutic advantage [12].

5. Effect of Tamoxifen on Cycle Dependent Chemotherapy Drug Activity

From the above narrative, one can clearly deduce that Tamoxifen and other hormone receptor regulators will have a detrimental and deleterious effect on the cytotoxic function of cancer chemotherapy drugs, eventually compromising their therapeutic benefits.

On the other hand, it has been postulated that estrogen may enhance the cytotoxicity of chemotherapy drugs used for breast cancer treatment by acting as an additive or sensitizing factor by increasing percentage of "S" phase of cycling cells [13].

6. Concurrent or Sequential Tamoxifen with Chemotherapy-Clinical Studies (Table 1)

The debate of concurrent or sequential use of hormones, especially Tamoxifen and chemotherapy, is reasonably settled. Clinical studies (**Table 1**) indicated that due to the cytostatic effect of Tamoxifen and other associated hormones used for cancer treatment, it is better given sequentially and not concurrently. As discussed above, concurrent usage of hormones is likely to compromise the cytotoxic effect of cancer chemotherapy, which is dependent on cycling cells especially on the "S" phase component.

Table 1. Chemotherapy—hormone therapy; concomitant or sequential clinical studies.

Author	Type of study	Conclusion
Gradishar W, *et al*. (2006) [2]	Analysis: multiple prospective study	Sequential recommended: Tamoxifen & Anastozole Combo is better
Baum M, 1988 [3]	Global multi-centric prospective	Adjuvant Tamoxifen on pre & postmenopausal Advantage: No comment on CON V SEQ
Pritchard KI, 2008 [14]	Review & Reflective	Hormone with chemo; CON V SEQ: yet a matter of debate
Albain K, *et al*. 2002 [15]	Prospective & Randomized	Should be "sequenced not concurrent"
Pico C, *et al*. 2004 [16]	Trend in favor of sequential	Trend in favor of sequential
Bedgonetti D, *et al*. 2011 [17]	Prospective & Randomized	No diff: CON V SEQ: Poor statistical power
Del Mastro L, *et al*. 2008 [18]	Prospective & Randomized	OS, DFS, Toxicity score: CON V SEQ = no difference Decreasing hazard of death-SEQ-group
Sideras K, 2010 [19]	Prospective & Randomized	Post-menopausal node +, Oe + − > SEQ more effective
Early Breast Cancer Trialists Collaborative Group (EBCTCG) 2005 [20]	Prospective & Randomized 145,000 pt—15 yr FU	CT + SEQ Tam significantly better than CT + No Tam CON V SEQ − not recorded

7. Effect of Tamoxifen on Cycle Dependent Chemotherapy Drug Activity

Ionizing radiation used for radiotherapy is essentially independent of cell cycle or cycling cells. It damages cells indiscriminately, irrespective of their malignant, benign or normal physiological status. Ionizing radiation damages cells by both intracellular and extracellular events. They are known as "4R" *i.e*., repair, re-oxygenation, redistribution and repopulation. None of these effects are cycling cell dependent. The events happen after the cells have been irradiated and the 4Rs conjointly sum up the final outcome of radiation on cancer cells. Irrespective of whether the cells are cycling or not. However, in cycling cells, phases G2 and M are relatively more radiosensitive; likely due to a higher number of target sites, prone to get damaged from ionizing radiation. As far as DNA strand break, that causes cellular radiation effects that are similar on individual DNA strands irrespective of the phase of the cell cycle [21].

8. Effect of Tamoxifen on Radiation Effects on Cancer Cells

As Tamoxifen increases percentage of G0/G1 phases, it will have a neutral impact on the cytotoxic effect of ionizing radiation. On the other hand, Tamoxifen also increases G2/M phases by blocking progress. It increases the number of apoptotic cells which are more radiosensitive than other phases likely due to the increased number of targets. Thus, the final outcome is a positive balance in favor of Tamoxifen concurrently administered with radiotherapy, which is likely to act as a radio-sensitizer [22].

9. Concurrent or Sequential Adjuvant Therapy of Tamoxifen and Other Hormones with Radiotherapy (Table 2)

Sequential or concurrent hormone therapy with radiotherapy for breast cancer is currently being debated. With the laboratory experimental clinical trials, the balance is in favor of concurrent usage of Tamoxifen with radiotherapy. It is important to get the best timing for hormonal adjuvant with radiation therapy, since hormones are

Table 2. Adjuvant concurrent vs sequential hormone therapy with radiotherapy: clinical studies and clinical study review.

Author	Type of study	Conclusion
Harris EE, *et al*. 2005 [24]	Retrospective 278 patients	No clinical impact on cosmesis, complication either modality: No comments on CON V SEQ
Azria D, *et al*. 2005 [25]	Commentary (retrospective data)	"Concurrent" increases subcutaneous and pulmonary fibrosis
Azria D, *et al*. 2004 [26]	Retrospective 147 patients	"Concomitant"-Tamoxifen (Tam) increases sub-cut breast fibrosis in hypersensitive patients
Whelan T, *et al*. 2005 [27]	Editorial review	SEQ or CON: yet to be resolved Randomized trial recommended
Pierce LJ, *et al*. 2005 [28]	Prospective randomized, 309 pts	No difference in adverse effect, local or systemic recurrence RT + TAM or RT only
Ismail SS, *et al*. 2013 [29]	Prospective 160 patients	No difference RT + CON or SEQ
Bentzen SM, *et al*. 1996 [30]	Retrospective 84 patients	Increase in lung fibrosis in CON Group
Ishitobi M, *et al*. 2009 [31]	Retrospective 264 patients	No difference between CON and SEQ Group
Tsoutsou PG, *et al*. 2010 [32]	Review	May be given CON or SEQ (RT) Combination of Tamoxifen and Letrozole recommended
Ahn PH, *et al*. 2005 [33]	Retrospective 495 patients	CON did not affect local control No observation on cosmesis and toxicity
Koc M, *et al*. 2002 [34]	Prospective 111 patients	RT + TAM V RT + 0: Tele cobalt RT Significant risk of lung fibrosis. No comment on CON V SEQ
Cecchini MJ, *et al*. 2015 [35]	Literature review	SEQ supported due to increase in lung fibrosis in CON treatment
Munshi A, *et al*. 2011 [36]	Randomized prospective	Results awaited (major study)

given for a period of 5 to 10 years. It is possible that minor but avoidable side effects from either choice might magnify to be a major clinical issue over a prolonged treatment period. **Table 2** lists clinical and experimental findings to assist in developing a consensus of optimal use of hormones with radiotherapy for breast cancer patients.

10. Effect of Surgery on Cancer Cell Kinetics

Hippocratic physicians from the 5th century BC through 7th century AD believed that ulcerated breast lesions were likely to recur more aggressively if resected, than those which did not present with ulceration [23]. Even though the conventional wisdom had been not to operate on cancerous breast lesions to avoid faster recurrence and spread, with the advent of anesthesia, surgical techniques became wider and more extensive without any real benefit for either relapse or survival. Several clinical studies did indicate that post-surgical residual cancer and dormant cancer cells are activated following surgical excision, growing faster and metastasizing more widely with virulence (**Table 3**).

A wide range of experimental research indicated the rapid growth of lung metastasis following resection of leg sarcoma [3], increased vascularity and reduced apoptotic cells in post-colectomy hepatic metastasis in rats [14]. Surgical trauma's responsibility for post-mastectomy recurrence and spread was reiterated by mathematical models in animals [15]. There was also post-surgical spiking of the labeling index (LI) in experimental tumors [16], post-surgical increase in residual tumor size, progression to proliferative phase and synchronization to sensitive phases in experimental system [17] (**Table 3**, **Table 4**). Experimental studies also indicated the essential role of tumor stroma in carcinogenesis, prevention of neoplastic development and functional dedifferentiation of breast cancer cells into normal ductal growth [18] [19] (**Table 4**). As an important extra-cellular effect of Tamoxifen, irrespective of estrogen receptor status, Tamoxifen interacts with the stromal-fibroblasts of human breast cancer and induces production of TGF beta1 (TGF B family), which is a novel receptor-independent action of Tamoxifen. This function of Tamoxifen is a potent inhibitor for the epithelial cell cycle, hence progression and growth of breast cancer [53] [54].

Several clinical and clinicopathological studies indicated: cytoreductive surgery for testicular tumors, enhanced tumor progression [20], and post-surgical accelerated growth of metastasis in non-small cell lung cancer [22]. Improved survival and relapse following Laparoscopic cholecystectomy compared to open cholecystectomy

Table 3. Clinical, clinicopathological and experimental studies: surgery + concomitant versus Sequential use of hormone in treatment of cancer patient.

Author	Type of study	Conclusion
Demicheli R, *et al.* 1997 [37]	Experimental Mathematical Model	Two peaks after resection. Surgical trauma, essential to manifest the peaks of recurrences
Peters CFJM, *et al*. 2006 [38]	Experimental & Clinicopathological	After partial liver resection, residual tumor shows aggressive and faster recurrence
Schatten WE, 1958 [39]	Experimental	Rapid growth of large number of latent pulmonary metastasis after removal of primary in leg
Lange PH, *et al.* 1980 [40]	Retrospective Pathological & Serological	Tumor progressed after cytoreductive surgery: clinical trial urged. Post resection recurrence of testicular tumors
Demicheli R, *et al*. 2008 [41]	Review & Critical Appraisal	From tumor dormancy to surgery driven enhancement of growth and metastasis likely
Baum M, *et al*. 2005 [42]	Review & Critical Appraisal	Surgery induced angiogenesis and proliferation of distant dormant micro-metastasis
Lacy AM, *et al*. 2002 [43]	Randomized Prospective	Laparoscopy assisted colectomy is superior than open colectomy; Morbidity, hospital stay, recurrence and Ca-related death, less: due to less surgical tissue damage
Mitsudomi T, *et al.* 1996 [44]	Retrospective	Post-surgical accelerated growth of undetectable residual cancer and dormant cancer cells
Gunduz N, *et al.* 1979 [45]	Retrospective	Post-surgical increase in residual tumor size; conversion of G0 to proliferative phase; synchronization of sensitive phases
Fisher B, *et al*. 1983 [46]	Experimental	Labelling Index (LI) peaks 3 days after tumor excision; Perioperative chemotherapy recommended

Table 4. Biological changes in post-operative environment: clinical and experimental findings.

Author	Type of study	Conclusion
Maniwa Y, *et al*. 1998 [47]	Clinical & Experimental	Disruption of angiogenesis suppression, induction of growth of dormant micro-metastasis due to post-op increase in VEGF
Ikeda M, *et al*. 2002 [48]	Clinical	Peri-gastrectomy serum concentration of VEGF, sP-selectin, v WF, involved in angiogenesis, tumor-platelet adhesion, tumor-endothelial cell adhesion factors; surgical intervention enhanced tumor growth and metastasis
Tagliabue E, *et al*. 2003 [49]	Clinical-operative specimen	HER2 over-expression by breast ca cells plays in post-surgery stimulation of growth of breast cancer cells
Wu FP, *et al*. 2003 [50]	Prospective Patho-physio, Cancer v normal tissue, 16 specimens	Local VEGF increase & endostatin decrease; physiological response to surgery, with or without cancer
Mitsudomi T, *et al*. 1996 [44]	Retrospective Clinical (197 cases)	Concluded residual tumor cells had accelerated growth after surgery
Maffani MV, *et al*. 2004 [51]	Experimental	Important role of tumor stroma indicated; further extensive study recommended
Maffani MV, *et al*. 2005 [52]	Experimental	Rat mammary gland tumor stroma prevents neoplastic development and encourage normal ductal growth from grafted epithelial cancer cells

suggests surgical trauma or lack of it is a responsible factor [24], as are disruptions of tumor-host homeostasis and activation of dormant cells [25], etc. Clinical and experimental studies indicated, post-operative increase in VEGF and a decrease in endostatin [27]. VEGF-induced angiogenesis stimulates growth of micro-metastasis [25] [26]. Post-gastrectomy increase in angiogenic factor, tumor-platelet adhesion factor, and tumor-endothelial cell adhesion factor influence tumor growth and metastasis [26] and post-surgical over-expression of HER2, stimulating cancer cell growth [35] (**Table 4**). Illustrated **Figure 1** and **Figure 2** indicates a possible existing dynamic tumor-host homeostasis (anticipate inherent changing balance by mutual consent) and results of disruption caused by surgical excision of the tumor, initiated by the "Substrate Vacuum" *i.e*., loss of the tumor mass, loss of stromal control on tumor growth and reduction of metastasis.

11. Effect of Tamoxifen on Surgery Induced Changes in Cancer Cell Kinetics

Having extensively reviewed clinical, clinic-pathological and experimental findings, the changes induced by Tamoxifen in post-operative surgical environment is getting clearer. The result of surgery alone in patients with cancer is essentially undesirable, except very small tumors which are yet to develop; a form of biological dynamic symbiosis between the tumor and host environment.

Strong evidences are now available to support the 2500-year-old Hippocratic dictum, not to operate on breast lesions which have ulcerated, because it regrows fast and spreads faster. We have enough striking evidence (**Table 3**, **Table 4**) to support the notion that postoperative loss of homeostatic balance between the tumor and the noncancerous elements, especially the stroma, induces residual cancer regrowth faster; micro-metastasis and dormant cancer cells are reactivated, changing to fast-proliferating, fast-metastasizing invasive cells. Surgical loss of stromal elements of the tumor, which commands significant influence on suppression of tumor proliferation, also negatively affects post-operative tumor control.

Hormones, *i.e*., Tamoxifen, which arrest cycling cells at G0 and G2 phases from progressing to G1 and M phases, respectively, are less likely to be affected by the post-operative perturbation of tumor-host homeostasis that induces accelerated tumor growth and metastasis. Thus perioperative use of Tamoxifen will prevent or reduce surgery induced accelerated residual tumor proliferation and metastasis. Also Non-hormonal control of Tamoxifen, tumor proliferation, with the help of tumor and normal stromal components, that aids retarding post-operative tumor recurrence and proliferation.

12. Recommendation of Sequencing Hormones with Other Therapeutic Agents

To rationalize sequencing Tamoxifen-like hormones, with other modalities, one needs to consider pharmacokinetics and pharmacodynamics of the hormone. Tamoxifen peaks at around 5 hours after a single oral use. To achieve a steady state plasma concentration, it takes 4 weeks, while on daily oral intake. The biphasic decline of

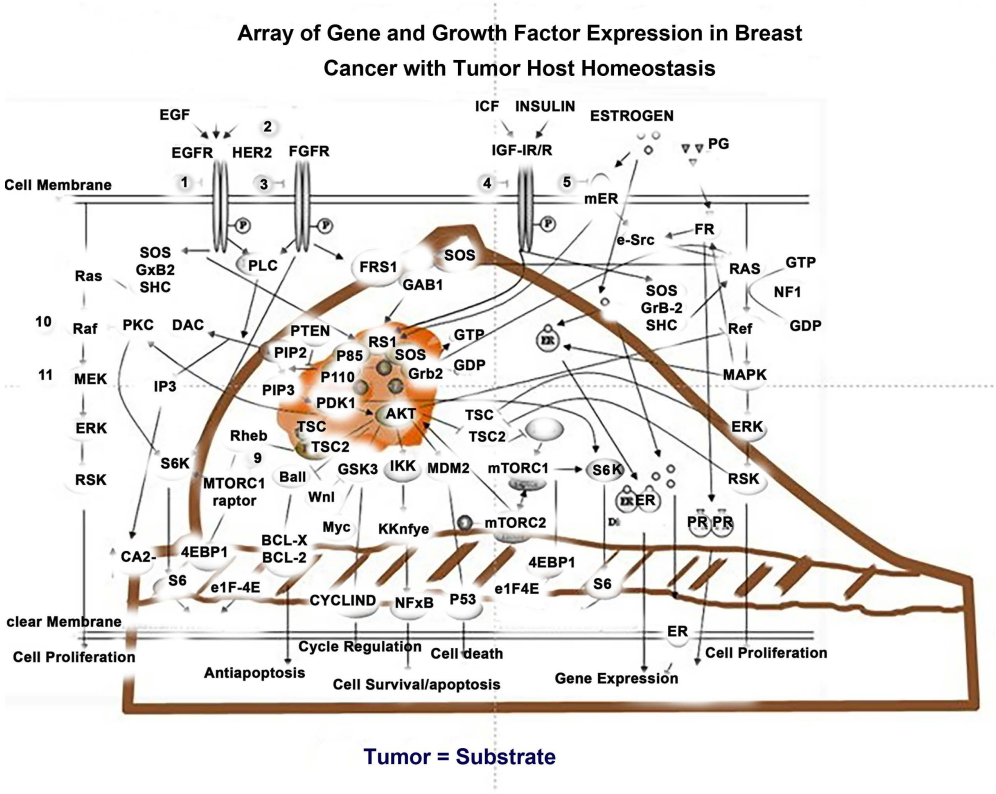

Figure 1. Array of gene and growth factor expression in breast cancer with tumor host homeostasis.

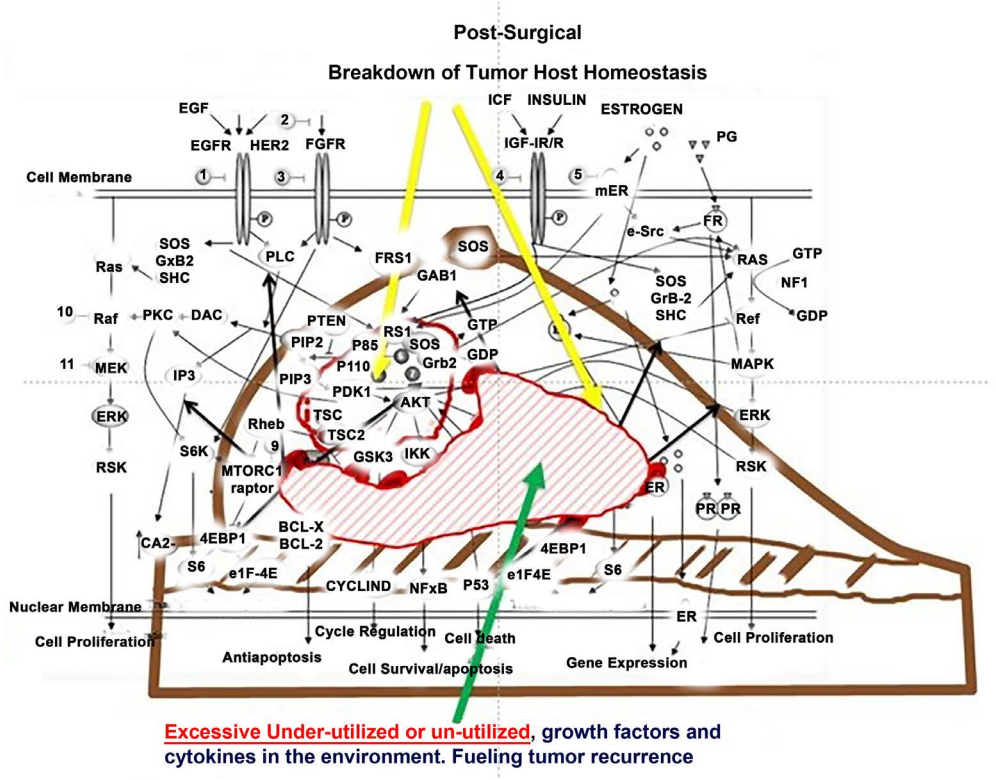

Figure 2. Post-surgical breakdown of tumor-host homeostasis and loss of stromal interaction.

plasma concentration pattern accounts for its biological half-life of 5 - 7 days. Sixty-five percent is excreted through feces over 14 days [54] [55].

Hence one needs to be aware of the pharmacology and biological effect of hormones used as adjuvants to a multimodality treatment plan, as well as the effect of Tamoxifen on the kinetics of the tumor itself and its impact on an individual agent's biological action on growth and metabolic kinetics of the targeted tumor. Thus an essentially pharmacological approach will impact the aggregate effect of multi-modality agents on the final outcome of hormonal adjuvant.

12.1. Tamoxifen and Chemotherapy

Tamoxifen and, for that matter, other cytostatic anti-hormonal agents, it is apparent from the above overview, act against the cytotoxic effect of chemotherapy drugs and would compromise the therapeutic benefit of chemotherapy. Tamoxifen should be stopped at least 7 days before commencement of chemotherapy. It needs to be resumed soon after (1 - 3 days), depending on chemotherapy drug elimination rate, and as soon as the prescribed cycle is completed. Hence, Tamoxifen and other cytostatic anti-hormones should be used sequentially with chemotherapy and not concurrently.

12.2. Tamoxifen and Radiotherapy

From the above overview, it appears that the biological impact of Tamoxifen on radiotherapy has been protective (detrimental) against radiotherapy due to G0/G1 block; also, cells in G0/G1 phase are relatively resistant to DNA damage due to a higher ability to repair. On the other hand, Tamoxifen induced G2-M delay/block has a sensitizing effect on radiotherapy, likely due to a higher number of target sites. The composite effect of Tamoxifen and, for that matter, aromatase-inhibitors have moderate radio sensitizing benefit. Unfortunately, this advantage is counterbalanced by the excessive fibrosis of lung and breast tissue (perhaps other sites too) post-concurrent Tamoxifen-radiotherapy treatment for breast cancer. This is an important fact to note in developing countries where radiotherapy is given mostly in tele-cobalt units, which do induce higher post-radiation fibrosis as compared to treatment by Linear Accelerators. Concurrent use of Tamoxifen with tele-cobalt generated radiotherapy, without any doubt, will exacerbate fibrosis of lung, chest wall, and the preserved breast after limited resection. Some individuals are sensitive to radiation, if detected, they should also not be given Tamoxifen concurrently with radiotherapy. A simple Lymphocyte-sensitive test can reasonably detect radiosensitive individuals. Otherwise some physical features, *i.e.*, people with blue eye, red hair, Irish-freckles, and people with tuberal sclerosis, xeroderma pigmentosa are known to be radiosensitive, should not be given tamoxifen concurrent with radiotherapy.

Thus, it is fair to use Tamoxifen sequentially with radiotherapy and not concurrently. Tamoxifen needs to be stopped 5 - 7 days (expected serum level to be lower than steady state, hence reduced bio-availability) prior to commencement of radiotherapy and resume couple of days after the last fraction of radiotherapy.

12.3. Tamoxifen and Surgery

From the above review, it is apparent that surgery does impact negatively on tumor control by accelerating proliferation of residuum and activating growth and metastasis of "dormant" microscopic non-proliferating tumor components. Tamoxifen and other cytostatic anti-hormone agents can inhibit to some extent this "blast" of post-operative tumor proliferative activities by retaining cells in the non-proliferative phase (G0/G1, G2-M). Hence, Tamoxifen and other cytostatic anti-hormones should be started 2 - 4 weeks prior to surgery and continued until there is a therapeutic reason to stop or pause.

Therefore, as soon as a tissue diagnosis confirms malignancy of the breast, even before the receptor results are available, it is recommended that Tamoxifen or Letrozole in post-menopausal patients be started to control tumor growth. It can be stopped if the receptors are reported to be negative, as and if they are available. The non-hormonal extra-cellular activity of Tamoxifen and Tamoxifen-stromal interactive-influence on suppression of cellular proliferation will also have a positive impact.

13. Limited Resource Countries-Optimizing Adjuvant Tamoxifen (Other Such Hormones Used for Adjuvant Treatment for Breast Cancer)

In developing countries, where resources are scarce and there is delay in getting the desirable surgery, a delay in

obtaining chemotherapy agents, a delay or non-availability of radiotherapy or even unavailability of "receptor status"; it is prudent to start Tamoxifen for all premenopausal and Letrozole to postmenopausal patients with biopsy proven breast cancer, prior to any definitive therapy, unless there is a preexisting clinical condition that dictates otherwise. Tamoxifen is inexpensive and generally available even in the developing world. Its side effects are minimal and can be monitored by a general practitioner and periodically by Gynecologists, not necessarily a Gynecological-Oncologist.

In receptor "unavailable" cases, the tumor being "receptor positive" is around 75% in pre-menopausal and higher in post-menopausal cases [56]. The extra-hormonal, extracellular, anti-breast cancer activity of Tamoxifen should also be taken into account. This essentially ignored function of Tamoxifen does add to the anti-cancer activity, irrespective of "receptor status". The advantages of adding Tamoxifen to the armamentarium of breast cancer treatment far outweigh the disadvantages and risks in receptor "unavailable" cases [57] [58]. Tamoxifen does reduce recurrences in the affected breast and also in the contralateral breast with DCIS, following lumpectomy and radiation; irrespective of receptor status [59].

Specifically, it is worth reiterating that the majority of developing countries' radiotherapy is delivered by tele-cobalt treatment unit. Extra effort should be made to ensure that Tamoxifen is stopped 1 - 2 weeks prior to starting radiotherapy and resumes 2 days after the last fraction, to minimize soft tissue post-radiation fibrosis (by this period, serum levels would have been reduced to an acceptable steady state, appropriate for the need).

As far as sequencing of Tamoxifen and like agents (Letrozole) for chemotherapy and surgery is concerned, evidence suggests the sequencing may be carried out as per resource rich countries, recommended in this overview.

While the developing world pines for acquisition of high tech, high cost, complex technology, and expertise-dependent interventions, that remains a wishful desire. These may be common in the developed world, but are unlikely to be available in limited resource countries. At the same time, LRC miss the simple adjustment to practice that may impact them profoundly; appreciation of a simple management strategy like "Optimizing Tamoxifen Sequencing" for breast cancer, as well as other neglected biological phenomena like "utilization of diurnal variation of drug effectiveness" and simpler technology utilizing "hyperthermia" to enhance the oncotoxic effect of other modalities, the scope of inhalation chemotherapy, etc. There are many other examples whose aggregate effect may supersede the therapeutic impact of most modern complex technological advances for cancer management.

14. Conclusions (Figure 3 for Graphic Recommendation)

It is a standard practice to prescribe Tamoxifen for hormone receptor positive patients with breast cancer. Immunohistochemical assay for hormone receptor of breast cancer is expensive and beyond reach for most "resource limited" regions, where eighty percent of world's population lives. However, the benefit of using Tamoxifen as an adjuvant treatment for "receptor unknown" cases of breast cancer far outweighs the dangers of recurrence and spread. Tamoxifen is far more readily available in resource limited country than the availability of immunohistochemical test. Hence, it is advised that irrespective of the "receptor status" all women with breast cancer can be placed on Tamoxifen or like, as long as there are no preexisting risk factors like, history of coagulopathy, DVT, diabetes, extensive varicosity etc.

It is suggested that as soon as histopathological diagnosis of primary breast cancer is confirmed, patients should be started on Tamoxifen (Letrozole for postmenopausal). Then, the plan should be amended when or if the receptor status becomes available.

Patients should continue Tamoxifen throughout the course of planned surgical management.

If radiotherapy is planned, Tamoxifen should be stopped 7 days prior to starting radiotherapy. If the patient is found to be radiosensitive on a Lymphocyte sensitivity test (a simple, but very useful test) or the patient has physical features of radio sensitivity *i.e.*, blue eyes, red hair, skin with excessive freckles; then there should be restrictions. Even mixed races with above features would have these restrictions. If suspended, then Tamoxifen should be started on the last day of radiotherapy. Otherwise Tamoxifen should be continued throughout the course of radiotherapy.

If chemotherapy is planned; Tamoxifen should be stopped 7 days prior to starting of chemotherapy and resume 2 days after the last infusion of chemotherapy. By that time, the effective role of chemotherapy would have waned.

Patients who are placed on Tamoxifen should be regularly checked by both an oncologist and gynecologist.

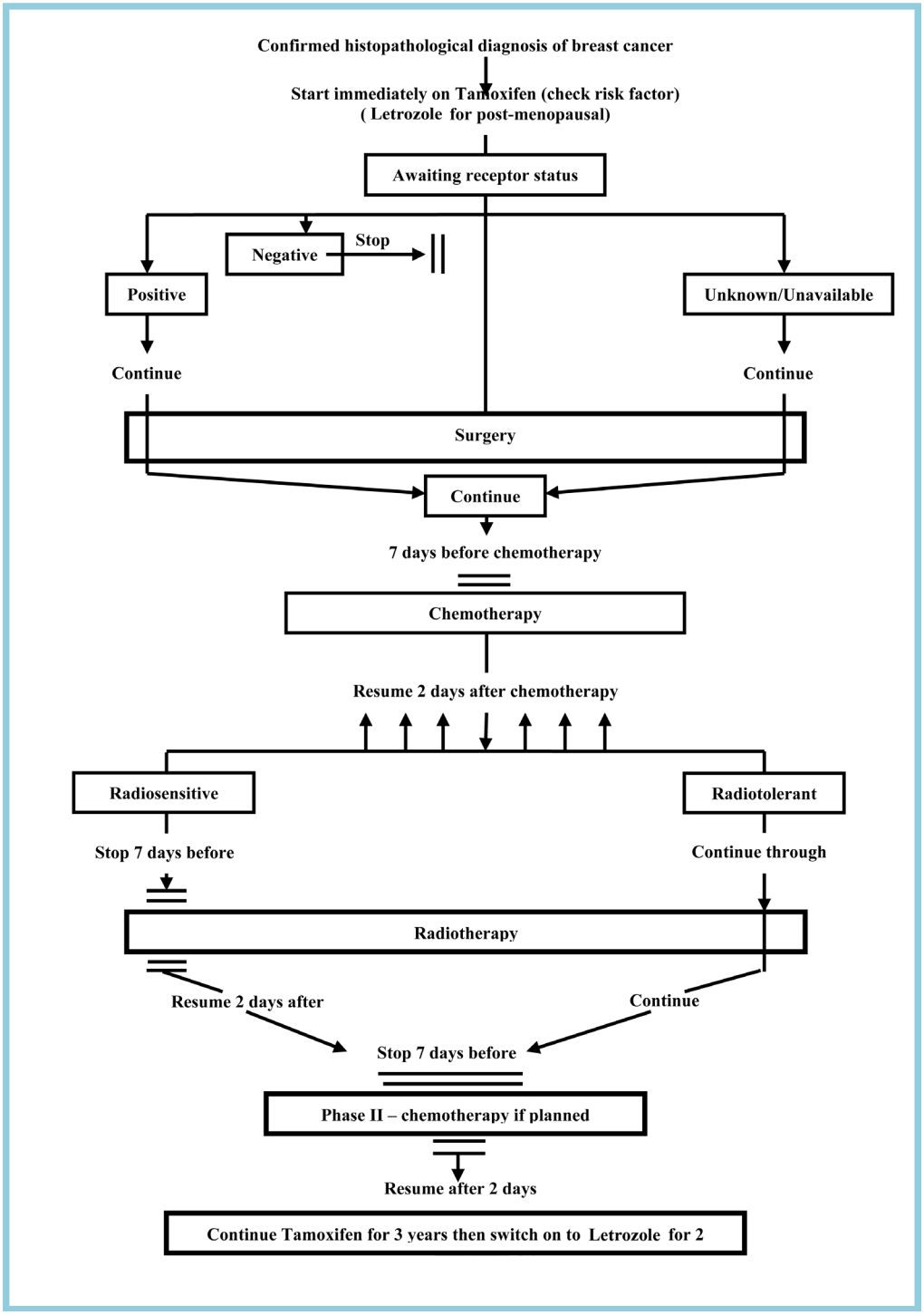

Figure 3. Comprehensive schema of Tamoxifen (Letrozole) during multimodality therapy and follow-up.

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