Lung Perfusion Imaging and Tc^{99m}-Macroaggregated Human Serum Albumin

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ABSTRACT–Lung perfusion scanning, invariably combined with ventilation studies provides a reliable and non-invasive mean to diagnose lung related pathologies despite the availability of modern techniques such as angiography, magnetic resonance imaging, magnetic resonance angiography, and helical (spiral) computed tomography. The technique involves the generation of images by radiations emitted from radioisotopes introduced in to the lungs. Various radiopharmaceuticals have been proposed and designed to incorporate Tc^{99m} in to macroparticulate form for lung perfusion imaging. However, most of these have associated difficulties such as reproducibility of the product with regards to particle size distribution and poor elimination from the lung capillary bed. Tc^{99m} macroaggregated albumin (Tc^{99m}-MAA) is used extensively for clinical lung perfusion imaging and is considered as the radiopharmaceutical of choice. It is non-toxic, safe, and being biodegradable, is easily eliminated from the lung capillary bed by proteolytic enzyme metabolism and by mechanical forces due to lung movement.

Keywords–Tc^{99m}-Macroaggregated albumin, Lung perfusion imaging.

Pulmonary nuclear medicine has been dominated by the techniques that visualize and assess the distribution of pulmonary ventilation and perfusion.[1] Thromboembolism in the lungs is potentially fatal pathological condition and continues to be the major indication for radionuclide lung scanning.[2-4] When pulmonary embolism is detected, the significance of diagnostic imaging is to direct and validate the treatment that is highly effective but not without complications[5]. For certain and accurate diagnosis of pulmonary embolism, pulmonary angiography still remains the reference standard. However, being invasive and expensive, the technique is not universally available. More recent advancement in this area of diagnosis is helical (spiral) computed tomography as the initial screening test for pulmonary embolism.[6,7] The technique involves imaging with intravenous contrast medium, and is considered to be minimally invasive. However, inconclusive scans and higher radiation dose to patients remain the limitations of this modality.[8] D-dimer assays have high negative predictive value whereas magnetic resonance imaging magnetic resonance angiography and electron beam computed tomography need future studies for specific validation.[9-12] Lung perfusion

imaging in association with ventilation studies still remains the most commonly employed screening technique for pulmonary embolism and involves generation of images by radiations emitted from radioisotopes introduced in to the lung field. The major advantage of all the radionuclides used in pulmonary studies is that when images of total lung fields are taken, regional function data are obtained.[13] The radionuclides and methodologies presently used are listed in Table I.

Ventilation Techniques

Ventilation studies of the lungs indicate the presence of any airway obstruction. Presently, three commonly employed methods of performing a ventilation study include; one that utilizes xenon gas (Xe^{133}), the second involves the use of radioactive krypton gas Kr^{81m} and the last is performed with radio-aerosol, usually the radio-aerosol of Tc^{99m}-HSA (Table I). Ventilation study with Xe^{133} consists of an image taken after inhalation of a single breath followed by serial imaging. After a breathing period of 3-5 minutes, the washout study is most reliable and sensitive to detect the slow compartments. Radio-aerosols also provide information regarding regional ventilation.[14,15] These consist of small particles that deposit by sedimentation and impaction.[16] Ventilation studies using radio-aerosol is gaining popularity and has extensively been reported in various diagnostic studies.[17-19] In order to observe

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Table I -*Radiopharmaceuticals Used in Lung Scanning*

Function	Disease condition
Perfusion studies	
i. Tc^{99m}-human serum albumin Macroaggregated Microspheres	Embolism detection Effect of ventilation disturbance.
ii. Tc^{99m}-pertechnetate	
Ventillation studies	
i. Radioactive gases Xe^{133} Kr^{81m}	Detection of airway diseases Embolism detection Detection of airway diseases
ii. Tc^{99m} aerosols	Bronchogenic carcinoma
Parenchymal studies	
i. Ga^{67}- citrate	Pulmonary infarction
ii. Tc^{99m}-DTPA aerosol	Bronchogenic carcinoma Interstitial lung disease
iii. Tc^{99m}-pyrophosphate	Parenchymal calcification

the various parameters interfering with ventilation procedure, a study has been carried out to evaluate changes in the distribution pattern of Tc^{99m}-HSA radio-aerosol in asthmatic patients before and after corticosteroid therapy.[20] The results revealed that a week long steroid therapy improves the bronchial obstruction in asthmatic patients and hence a homogeneous distribution pattern of radio-aerosol is achieved. The addition of a spacer has also been shown to improve pulmonary aerosol deposition from a jet nebulizer during mechanical ventilation.[21]

Perfusion Lung Imaging

The term perfusion lung imaging clinically refers to imaging of the pulmonary artery perfusion distribution in the lungs.[22] It is a non-invasive method for the evaluation of pulmonary arterial blood flow. The underlying principle of perfusion imaging is the use of particulate radiopharmaceuticals and their distribution pattern in the pulmonary arterial bed that is considered to be pulmonary blood flow dependent (Table II). The technique is based upon assumption that when introduced into the biological system, the particulate radiopharmaceutical gets evenly distributed in the blood before reaching the pulmonary artery.[23] Influenced by various factors, such as

Table II -*Perfusion Scan Principle*

i.	Radiolabelled albumin particles are too large to pass through the pulmonary artery capillary network.
ii.	Particle distribution is proportional to pulmonary artery distribution at the time of injection.
iii.	Anything that alters pulmonary artery distribution may result in abnormal scan.
iv	Images are relative to pulmonary artery distribution and not to the absolute blood flow.

haemodynamics and gravitational force, the particles are almost completely extracted from pulmonary circulation in a single passage through the lung. The extraction efficiency of the order of more than 90% has been achieved.[24] The most important parameters, however, are the particle size distribution and mixing with the blood. This directly interferes with the mechanism of localization wherein particles with size larger than the diameter of the smallest vessels in the arterial network (7-10 μm) are trapped over there.

Pulmonary embolization, or the dislodging of small clots that usually originate in the deep venous system of the lower extremity, is clinically a difficult diagnosis.[25] The normal lung perfusion imaging study virtually eliminates the diagnosis of pulmonary embolization.[2] Conversely, an abnormal perfusion lung image is formed in almost all diseases including pulmonary embolization, perfusion changes secondary to malignancies and emphysema. Over the last decade, physicians have set parameters as visualized on the lung images to improve the specificity of lung imaging in the diagnosis of pulmonary embolization. Ventilation study with Xe^{133}, or Kr^{87m}, if performed in conjunction with the lung perfusion images, improves upto 90% of the sensitivity of the lung perfusion image.[3] As a general rule, normal ventilation is usually found in the region of pulmonary embolization. Lung perfusion imaging in conjunction with ventilation imaging has added a non-invasive component to the proper evaluation of patient with bronchitis and obstruction forms of COPD.[26] Moreover, this technique has shown promise over other techniques in the diagnosis of chronic airway diseases and in the prediction of postneumonectomy pulmonary function using perfusion lung scanning.[27,28] In a recently published report, Xe^{133} ventilation and Tc^{99m}-MAA perfusion studies have been employed to assess irreversible long term pulmonary impairment after adenovirus type-7 pneumonia.[29]

Bronchogenic carcinoma, the most common form of lung carcinoma, causes a disease or absence of pulmonary blood flow to the affected bronchial segment either by compression or by reflex action.[30] Lung perfusion remains in the total lung field to predict whether the patient will become a respiratory cripple if the involved carcinomatous portion of the lung is intimately involved. Since the lung is involved with cardiova - scular dynamics, congenital or acquired heart disease causes profound changes in pulmonary arteriole physiology[31,32] Thus, lung perfusion imaging is an indirect indicator and may be used to follow the results of corrective surgery. Moreover, the lung perfusion image in conjunction with patients clinical status, chest X-ray findings, and the results of a nuclear ventilation study constitutes an integral part of the diagnostic

criterion.

A recent application of Tc^{99m}-MAA is in the measurement of pulmonary vascular granulocyte pool, which gets expanded in many conditions associated with systemic inflammation.[33] The application of lung perfusion scan using Tc^{99m}-MAA has also been evaluated for its usefulness in hepatopulmonary syn - drome.[34] An interesting study has been reported wherein distribution ratio of Tc^{99m}-MAA in tumor to non-tumor tissue has been described to correlate well with other techniques.[35] Lung perfusion SPECT using Tc^{99m}-MAA has also been used in predicting post-operative pulmonary function in lung cancer.[36,37] In a recently published report, promising results have been obtained to estimate regional lung function in interstitial pulmonary disease by SPECT using Tc^{99m}-MAA.[38] The value of perfusion scintigraphy has also been successfully elaborated as a screening test for children who have suffered several recurrent episodes of localized pneumonia.[39] In a later study, Soler *et al.* (1997) has proven the usefulness of Tc^{99m}-MAA perfusion study to evaluate the severity of bronchopulmonary dysplasia in a study group of ten children.[40]

Study of lungs by colloidal particles

Particles of colloidal dimension have frequently been used to study the lungs either by intravenous administration (lung scanning) or by nebulization (lung deposition and clearance). Particles in the size range 10-20 μm diameter are filtered out by the lung capillaries, with high extraction and primarily to the alveolar segments[41]. The human lung is believed to have about 2.8×10^8 alveoli and 2.8×10^{11} capillary segments.[42] Pulmonary artery blockade was first visualized by scintillation scanning using Au^{198} adsorbed on 50 μm carbon particles and Ag^{111}- and Hg^{208}-labeled ceramic particles.[43-45] These radio-pharmaceuticals are particulate in nature and contain particles ranging in size from 15-70 μm. A wide variety of colloidal systems have since been examined as lung scanning agents including Tc^{99m}-iron hydroxide aggregates and sulfur colloid macroaggregates.[46,47] Illum *et al.* (1984) reported the use of polystyrene particles labeled with I^{131}-rose bengal to investigate effects of particle size and particle shape on lung deposition.[48] Polystyrene microspheres of 15.7 μm mean diameter and DEAE-cellulose microspheres 40-160 μm diameter, at a dose of about 10^8 particles, are rapidly extracted and lodged in the lung capillaries. Non biodegradable polystyrene and teflon particles generated as monodispersed systems by spinning disc generator and labeled with Tc^{99m} are now widely employed in studies on lung clearance by mucociliary and cough mechanisms and the effects of drugs and disease states. Cellulose fibers (nominal 5 μm in length) proved fatal causing blockade of the

main vessels to the lungs. However, due to the associated disadvantage of being non-biodegradable and hence, having an infinite retention in the lungs, these are considered as unsuitable for human use.

In the later studies, clusters of macroaggregates of albumin or single spherical particles of albumin such as microspheres and milli microspheres made from human serum albumin have extensively been reported in pulmonary imaging.[49-53] Davis (1986) indicated that the size range between 13.5 ± 1.5 μm diameter would be ideal.[54] However, this particle size distribution was too narrow for commercial production and proposed a range 10-20 μm (15 ± 5 μm). Such a system has large margin of safety, provided that the administrated dose (particles number) is very small in comparison to the number of capillary segments. The toxicity is related to the size; large particles (90 μm diameter) being more toxic as compared to the small ones.[54]

Macroaggregates of human serum albumin

Imaging with Tc^{99m}-labelled macroaggregates of human serum albumin is well established as a safe and reliable method of diagnosing pulmonary embolism.[55] Earlier studies were carried out using I^{131}-labeled MAA.[56] Reports have also been published to use In^{111}-labeled MAA for use as alternative tracer for combined perfusion-ventilation imaging.[57] However, since the advent of Tc^{99m}, macroaggregated human serum albumin labelled with Tc^{99m} has established itself as a radiopharmaceutical of choice for lung perfusion studies.[58,59] It provides a rapid and safe mean of confirming suspected massive pulmonary embolism prior to surgery.[60] Animal and human studies spanned over more than three decades demonstrate remarkable margin of safety in the procedure.[53,61,62] However, a report published by Renowden *et al.* (1990) showed that arterial oxygen saturation fell sharply and significantly following the intravenous administration of Tc^{99m}-MAA for lung perfusion studies that may prove fatal for the patients.[63] A subsequent study to assess these findings showed that majority of the 101 patients included in the study for perfusion scan with Tc^{99m}-MAA showed no significant fall in oxygen saturation.[64] If at all it occurred, it was related to the underlying disease rather than to the use of Tc^{99m}-MAA.

Biodegradation of albumin is an added advantage associated with the use of albumin based pulmonary diagnostic agent. The rate of breakdown of albumin (either in the form of macroaggregates or microspheres) depends upon the so-called "hardness" of the system which is related to the method of preparation and treatment (heating temperature, cross-linking agents etc). This is cleared rapidly from the lungs by enzyme

metabolism, aided by haemodynamic pressure and the mechanical forces originating from the movement of the lungs. [64] Total clearance from the lung field is generally seen within 24 hours. However, the rate of breakup of the microsphere and the loss of the label do not necessarily occur at the same rate.

Moreover, the commercial availability of ready-to-use "instant" MAA kit together with the availability of Tc ^{99m} in the form of generator system, has enabled most nuclear medical centres to use Tc^{99m}-MAA for lung scanning. Over the years, various techniques for the preparation of Tc ^{99m}-MAA have been proposed and reported in the literature. [52,53,65-68] However, these attempts have met many difficulties such as lack of reproducibility of the product with respect to particle size distribution and fragility of the aggregates.

Investigations have been continuing to simplify the method of preparation of MAA kit to overcome the above mentioned problems and with enhanced stability, labeling efficiency and lung uptake. [61,69-73] The authors have successfully attempted to simplify the methods of Lyster et al. (1974) and Al-Janabi et al. (1983) to produce an in-house, ready-to-use MAA kit .[68, 69] Our method of MAA preparation involves simple heating and does not involve the use of many chemicals, at pH 5.5 using PVP-30 to impart excellent stability and integrity on the aggregated HSA. [74] The In-house prepared kit has since been used in more than 100 patients for clinical investigation of various lung pathologies and has given good results in comparison with the results achieved with commercially
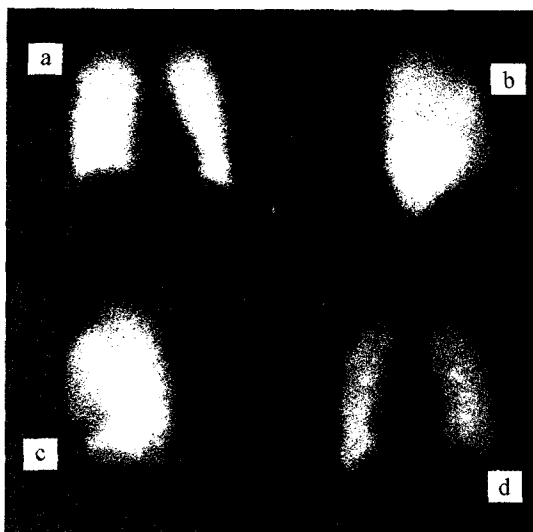

Figure 2–Photomicrograph of a section of lung two hours post injection of Tc^{99m}-MAA in a rabbit. The macroaggregates of HSA are seen entrapped within the alveolar septal vessels.

available kits (Figure 1). [75]

One important consideration in the preparation and supply of radiopharmaceuticals based on pooled human blood products such as HSA and fibrinogen, is the possibility of donor infection. To alleviate this problem, Perkin et al. (1994) have reported the production and development of recombinant human serum albumin (rHSA) microspheres tagged with Tc ^{99m} .[76] The animal studies using rHSA have given very encouraging results.

The size of macroaggregates and their number to be administered to the patients have remained debatable since the inception of particulate radiopharmaceuticals for lung perfusion imaging. [44,62,77] Histological studies reveal the entrapment of MAA during the first circulation in the pre-capillary vascular bed of the lungs (Figure 2). This is consistent with the facts that lung capillary diameter is 10-12 mm whereas the particle size of MAA ranges 10-90 mm. Diagnostic Tc^{99m}-MAA kit does not contain particles larger than 150 mm or smaller than 5 mm in diameter. [78] It is necessary to determine smaller or larger particles in Tc ^{99m}-MAA formulation with the size range of 5-150 mm in diameter.

Approximately 5 million particles are injected to the patients which block approximately equal number of capillaries in the lungs. Considering the haemodynamics and mechanical forces originating from movements in the lungs, the safety of MAA for human use has proven to be beyond doubt. [64] However, as the patients with respiratory insufficiency and pulmonary

Figure 1–Various views of a lung perfusion scan in a human patient using in-house prepared Tc^{99m}-MAA kit. The kit was lyophilized and used after two months storage in refrigerator. a) Anterior view at 20 minutes post injection, b) Right lateral view at 24 minutes post injection, c) Left lateral view at 26 minutes post injection, d) Posterior view at 28 minutes post injection.
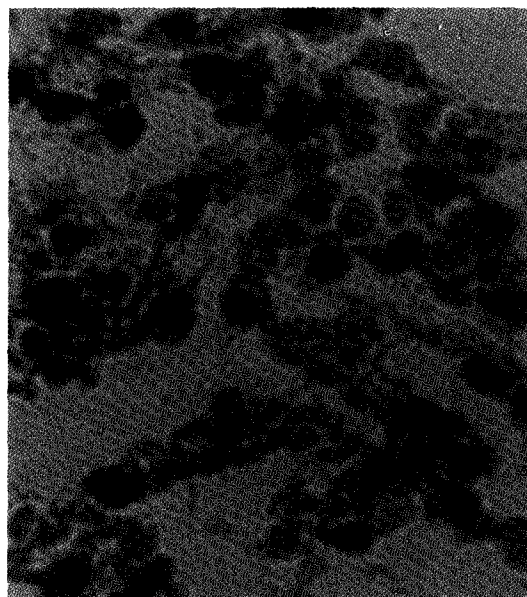
embolism and related pathologies are already distressed, the number of particles to be injected and their size distribution are still considered as critical factors. Both these parameters grossly affect the accuracy and reproducibility of the results. [79] Furthermore, the type of HSA used in the aggregation considerably influences the particle size of MAA apart from other factors such as concentration of albumin, pH and the type of the buffer used during aggregation process. Human serum albumin is a mixture of non-mercaptalbumin and mercaptalbumin.[62,80-84] Non-mercaptalbumin is composed of mixed disulfide type non-mercapalbumin with cystein or glutathione and oxidized type non-mercaptalbumin such as sulfinic and sulfonic states.[85-87] Aggregation of oxidized non-mercaptal - bumin have been found to contribute smaller sized particles in the MAA formulation, this being inappropriate factor for scintiscanning of lungs.[88]

Conclusion

Perfusion scintiscan elaborates the perfusion pattern of the lung capillary bed and when carried out alongside ventilation studies, together these provide an excellent non-invasive and simple mean to detect patterns of segmental perfusion deficits and ventilation defects. The primary indication for pulmonary perfusion and ventilation imaging is acute pulmonary embolism. Despite the emergence of various promising diagnostic modalities during the last decade for the evaluation of patients with pulmonary embolism, a standard diagnostic approach is needed to be developed. At present, perfusion scan still remains the method of choice and Tc^{99m}-MAA retains its position as the workhorse radiopharmaceutical for pulmonary embolism detection.

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