Original Article

# The Heat Denatured RBC, the Most Specific Technique for Spleen-Specific Imaging: Siriraj Nuclear Medicine Experiences: 2007 - 2014

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***Abstract***

**Objectives:** To detect a remnant of the spleen as well as the ectopic spleen in the patients who have undergone splenectomy and developed recurrent thrombocytopenia in order to localise residual splenic tissue for re-surgery, the most specific technique is the usage of denatured red blood cells, which has been studied and established in the Division of Nuclear Medicine, Siriraj Hospital and used for those patients as requested by clinicians since 2007. It will obviously be fruitful to share the efficiency and efficacy of the technique to clinicians for the benefit of the patients. Thus, the results obtained during 2007 - 2014 were reviewed.

**Materials and Methods:** The patient's blood was labelled with a radiopharmaceutical Technetium-99m (99mTc) in vivo-vitro giving 99mTc-Red blood cells (99mTc-RBC). The labelled RBC were then heat denatured to be spherocytes via a critical temperature of 50 ( 1 o C and re-injected into the patient. An imaging was performed through a gamma camera with low-energy, general purpose collimator. The images were interpreted by a Nuclear Medicine physician without knowing of clinical or laboratory data of the patients. The benefit of the denatured RBC technique was studied.

**Results:** From 2007 to 2014, there were only 7 patients and 1 normal subject. An average percentage of 99mTc-RBC labelling was 91.6, and that of the spherocytes obtained from the heat denatured RCB was 100 in nearly every preparation. In the imaging view for the normal subject, the spleen was clearly shown and would be used as 'control'. For most of the patients, their accessory spleens and/ or normal spleens were also obviously demonstrated.

**Conclusion:** The technique showed high percentage of radio-labelling as well as successful heat denaturation of RBC to provide enough sherocytes to identify the ectopic spleen as well as confirm the outcome of splenectomy of the patients. So, it is useful to identify the residual splenic tissue in the patients with recurrent ITP post-splenectomy. This most specific method for the spleen is now well established in the Division of Nuclear Medicine, Department of Radiology, Siriraj Hospital to provide benefits to the patients.

**Keywords:** Denatured red blood cells, Splenectomy, Spleen imaging.

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## Introduction

In the patients with hypersplenism status post splenectomy but had recurrent idiopathic thrombocytopenic purpura (ITP), residual splenic tissue is suspicious. There are many investigating options for this setting, such as ultrasonography, computed tomography (CT), scintigraphy with Tc-99m sulfur colloids, and Tc-99m denatured red blood cells. For anatomic imaging such as ultrasonography and CT, they give low specificity. For example, if soft tissue density nodule is found on CT image, it cannot be characterised to be splenic tissue or not[1].

Reticulo-endothelial cells (RE cells) have phagocytic property. They are located in few organs such as the bone marrow, the liver and the spleen. Their important function is to filter and clean the blood by trapping debris (particles), microorganisms, and abnormal or old blood cells. Distribution in the different RE-organs depends on a particle size. Small particles are trapped by the bone marrow and the liver. On the other hand, large particles are trapped by the spleen. According to their phagocytic property, we can use radiolabelled particles, such as Tc-99m sulfur colloids and Tc-99m denatured RBC to image these organs[2]. Since Tc-99m sulfur colloids have a relatively small particle size, the tracer was taken by the liver more than the spleen. Splenic nodule uptake may be masked by the intensed liver uptake and sensitivity is not satisfied. However, Tc-99m denatured RBC is prepared from normal red blood cells properly denatured to be spherocytes. This has a larger particle size, so most of them are exclusively trapped by the spleen and also known as the most specific imaging modality for the spleen[3,4].

In the Division of Nuclear Medicine, Siriraj Hospital, the technique was introduced to serve our clinicians in 2007[5,6]. Since it is delicate, it has been carefully adopted and slightly improved throughout the years up to now to establish a suitable in-real-life denatured red blood cells technique for spleen-specific imaging. If our clinicians realise its benefits to the patients and its availability in Siriraj Hospital itself, the request to serve their patients should be risen up.

## Materials and Methods

**Materials for labelling of red blood cells:** Stannous (Sn) kit, $Na^{99m}TcO4$ (Sodium pertechnetate) in normal saline solution (NSS), Heparin as an anticoagulant, and other materials including sterile syringes and needles, lead shields, test tubes.

**Materials for denaturation of red blood cells:** Falcon tubes (conical centrifuge tubes) - sterile and non-sterile, a water-bath with shaker and preset temperature of $50 \pm 1\ ^{o}C$, a centrifuge, and other materials including sterile syringes and needles, beakers, test tubes.

**Methods:** To prepare denatured labelled RBC, there are two sequential steps: RBC labelling with a radio-isotope and denaturation of the labelled RBC, as follows:

**RBC labelling[7]:** An in vivo-vitro technique: Reconstitute Sn kit with NSS as mentioned in its leaflet and inject it into the patient. After 20 - 30 minutes, draw blood of 10 ml into a heparinised syringe. Then, under a lead glass shielding, add 20 mCi of $Na^{99m}TcO4$ to the blood, mix well, and allow to stand for 5 minutes to form $^{99m}$Tc-RBC. Separate a small amount of labelled RBC aside for checking percentage of labelling. It was then centrifuged and separated packed RBC from plasma. Each

part was counted for radioactivity and calculated for percentage of radiolabelling to RBC.

**Denaturation of RBC[6]:** Transfer the labelled RBC into a sterile Falcon tube. Place the tube into a water-bath with a pre-set temperature of 50 ± 1 $^{o}$C for 20 minutes with periodical shaking. Most of the RBC were transformed into sperocytes in this stage. Wash the denatured RBC once and reconstitute with NSS. Collect a small amount of the blood for checking labelling percentage again. Prepare suitable activity in a 10-ml syringe ready for re-injection as follows: adults, 5.4 - 8.0 mCi (200 - 300 MBq) and children, 2.7 - 4.3 mCi (100 - 160 MBq). Before the injection, the denatured labelled RBC must be checked for the amount of spherocytes via the Wright stain (or an equivalent); it was done with the kind co-operation of the Faculty of Medical Technology, Mahidol University. The percentage of 80 or more is accepted.

**The patients in the study:** The study was done under the Siriraj Institutional Review Board with the Ethics Certificate Number of Si 085/ 2015.

Those requested by the clinicians of Siriraj Hospital for spleen imaging at the Division of Nuclear Medicine, Department of Radiology, and agreed to have a re-injection during 2007 to 2014 were included. The one who refused to have a re-injection of her own blood was excluded. In addition, a normal spleen imaging was performed by one volunteer who has never been undergone splenectomy.

The related historical data of the patients have been reviewed through their medical records and collected in case record forms which were kept confidentially.

**Principle of the technique:** Red blood cells of a patient are labelled with a radioisotope, Tc-99m pertechnetate ($^{99m}$TcO4-, Na$^{99m}$TcO4), via an in vivo-vitro method to get $^{99m}$Tc-RBC which will then be denatured to be spherocytes using a water-bath prior set to be 50 ± 1 $^{o}$C. They will next be re-injected into the patient and the scan is done under a gamma-camera with low-energy, general purpose collimator. The imaging is obtained at 30-120 minutes after radiopharmaceutical injection in anterior and posterior planar views. SPECT/CT imaging of abdomen was performed in selected cases.

## Results

During 2007 - 2014, 7 patients and 1 normal subject were included in the study with an age range of 5 - 70 years. Six of those 7 patients have undergone splenectomy prior to the scans.

**Percentage of RBC labelling:** An average percentage of 8 times of radio-labelling
(Tc-99m RBC) was 91.6.

**Percentage of spherocytes:** Almost 100 % of denatured RBC as spherocytes were obtained in every preparation as shown in Figure 1. However, some debris or fragments could also be found.

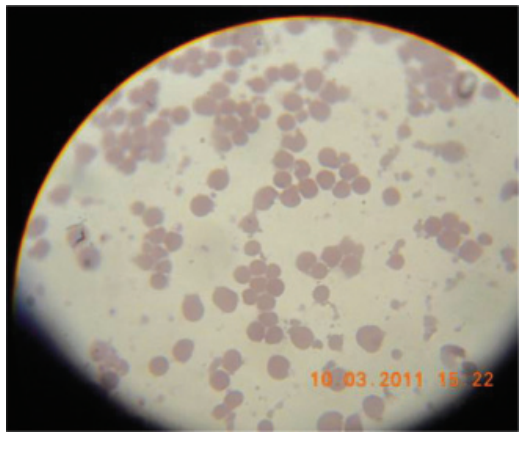


**Figure 1**. The denatured RBC as spherocytes (Wright staining).

**Imaging of the Normal Volunteer:** The anterior and posterior views of the spleen of the volunteer were shown clearly as control, Figure 2.

**Imaging of the patients:** The accessory spleens of the patients could be demonstrated in the images in nearly every case and pointed out by a Nuclear Medicine physician as shown in Figure 3 and Figure 4 as an example.

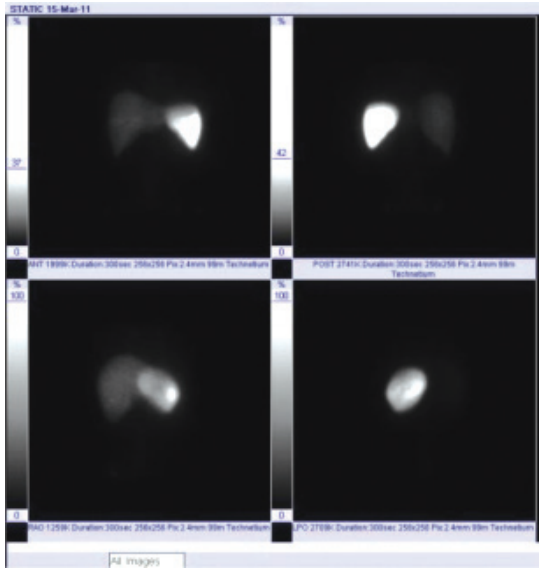

**Figure 2**. A normal spleen, anterior, posterior, RAO and LPO views shown by the denatured RBC technique.

## Discussion and Conclusion

It took quite a lot of energy for the team to set up this technique, since it has never been developed to be used in the Division of Nuclear Medicine, Siriraj Hospital or most of other hospitals in Thailand before. After care-

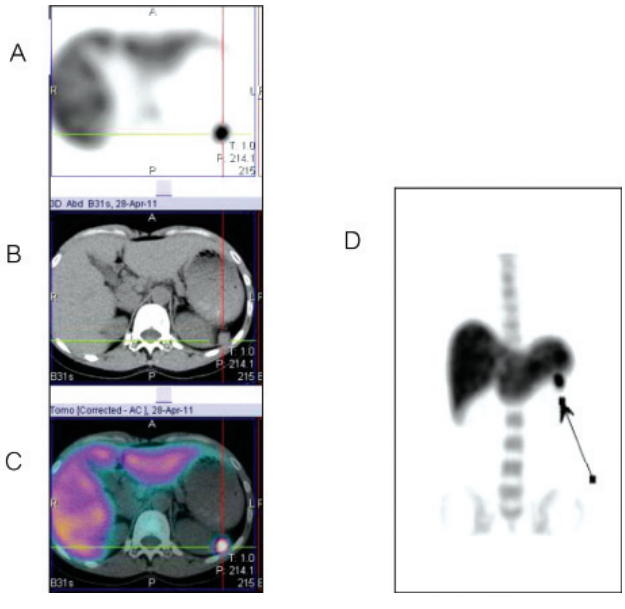


**Figure 3**. Axial images SPECT (A), CT (B), SPECT/ CT (C), and planar (D) of the patient with previous splenectomy showed a focal increased Tc-99m denatured RBC uptake at the splenic bed (an arrow), compatible with the splenic remnant.

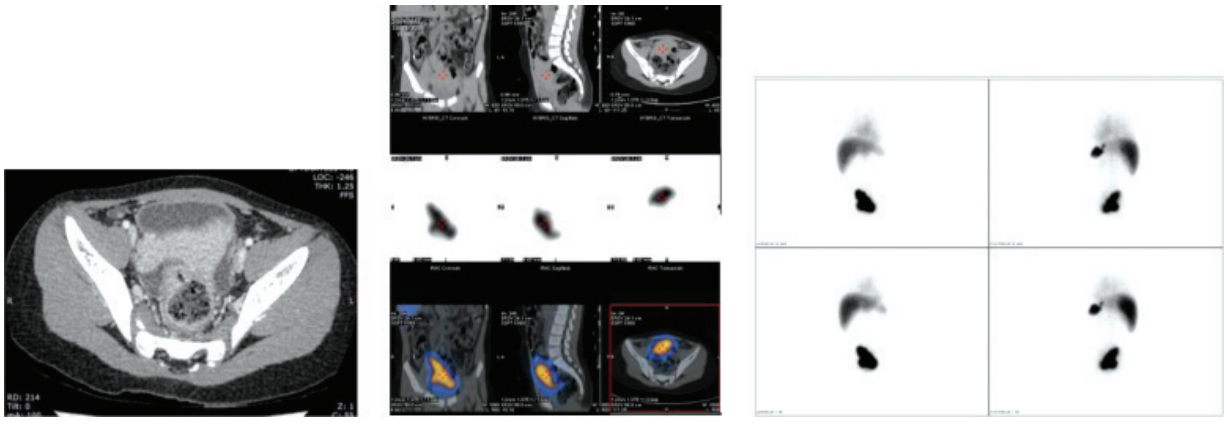

**Figure 4**. Images by CT (A), SPECT-CT (B), and planar (C) of the patient without previous splenectomy showed an ectopic spleen near the bladder.

fully reviewing papers related and consulting the known experts in Australia and UK, a technique was introduced with great care for our first patient. It was then slightly and delicately improved throughout the rest of the patients as requested 0 - 1 case per year, technically focused on the required temperature to denature red blood cells into spherocytes with enough numbers.

An in vivo-vitro method for RBC labelling was chosen giving a high percentage of radio-labelling, and the labelled RBC would then be denatured in vitro before reinjection. The second step, denaturation of RBC, is delicate. The desired temperature of a water-bath is critical especially inside the immerged tubes to make normal RBC become 'spherocytes', mainly and specifically destroyed at the spleen, not the liver. The launched technique gave satisfactory denaturation of RBC as required, though the excretion into the liver can be seen which may be due to RBC fragments occurred during denaturation.

The images in the spleen study obtained through the RBC denaturation technique developed in our Division of Nuclear Medicine showed satisfied results for both the patients and the normal volunteer. The study shows the clinical usefulness and advantage to identify the splenic remnants or ectopic spleen as well as confirm the outcome of splenectomy. If our clinicians learn that there is an effective spleen imaging served at Nuclear Medicine Division, more requests may be gained, obviously providing more benefits to the patients with recurrent ITP post-splenectomy.

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