# Selective Splenic Scintigraphy: A Very Simple Technique

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Summary. Selective splenic scintigraphy is specifically recommended for studying the spleen. A very simple "in vitro" method to label red blood cells with <sup>99m</sup>Technetium with concurrent termic denaturation is described. This technique makes it possible to obtain selective splenic scintigraphic images, with very few manipulations, when compared with other similar procedures.

#### INTRODUCTION

Techniques to label red blood cells (RBC) with radionuclides <sup>51</sup>Chromium (<sup>51</sup>Cr) and <sup>99m</sup>Technetium (<sup>99m</sup>Tc) have been used in several procedures in nuclear medicine. <sup>1-4)</sup> The physical characteristics of <sup>99m</sup>Tc emission favor its utilization in place of <sup>51</sup>Cr for various examinations. <sup>99m</sup>Tc-labeled-RBC is used among other application to study the blood volume, to localize gastro-intestinal bleeding, to determine the splenic sequestration and to obtain vascular

imaging.<sup>1,5)</sup> Selective splenic scintigraphy has supplied useful information in many clinical situations such as enlarged or atrophied spleens, accessory or ectopic spleens, space-occupying lesions and infarcts.<sup>6)</sup> Selective splenic scintigraphy has been obtained with denatured labeled RBC.<sup>16)</sup>

The <sup>99m</sup>Tc labeling of RBC requires a reducing agent, since this radionuclide seems to bind to the hemoglobin molecule at lower valences.<sup>8)</sup> One usual compound to reduce <sup>99m</sup>Tc, as sodium pertechnetate, is stannous chloride.<sup>2,9,10)</sup>

<sup>99m</sup>Tc-labeled-RBC may be obtained through "in vivo", 110 "in vitro", 9,10,120 or "in vivo/in vitro" assays. 13,140 "In vivo" methodologies may be employed for oral 150 or for intravennous 140 stannous chloride administration. The "in vitro" techniques lead to the obtaining of better product than "in vivo" ones. 20 RBC labeling through "in vitro" techniques may occur at different temperatures (room temperature, 37 and 50°C). 160

The "in vitro" procedures to label RBC with 99mTc.

**Table 1.** A very simple technique to obtain selective splenic scintigraphy with <sup>99m</sup>Tc-labeled and concurrent termic denaturated red blood cells.

- —Draw 1.5 ml of whole blood into a syringe with 0.5 ml of ACD anticoagulant (dextrose, sodium citrate and citric acid solution).
- -Add this mixture into a sodium-stannous kit diluted previously with distilled water.
- -incubate this mixture for 20 min at 50°C, with gentle shaking.
- -Add 0.5 ml of 99mTc solution and incubate the kit under the same conditions for another 10 min.
- -Allow the mixture to reach a proper temperature for reinjecting into the same patient.
- -Make the selective splenic scintigraphy 20 min after reinjection into the patient.

The percentage of 99mTc uptake by the RBC was always above 95%.

as described in the literature can comprise several steps, many manipulations<sup>7,14)</sup> as well as necessitating a substantial volume of whole blood.<sup>10)</sup> In the present work we propose a very simple "*in vitro*" methodology to obtain <sup>99m</sup>Tc-labeled-RBC with concurrent termic denaturation and its application in selective splenic scintigraphy is shown. This technique requires very few manipulations and only a small volume of whole blood.

## MATERIALS AND METHODS

Studies of selective splenic scintigraphy were performed, on eight dogs<sup>17)</sup> and afterwards on volunteer patients.

Selective splenic scintigraphy was obtained through the following steps: 2 ml of a mixture of whole blood plus anticoagulant (dextrose, citric acid and sodium citrate solution), in the proportion of a total volume of 4 to 1 of anticoagulant were all placed in a sodium-stannous kit, 18,19) previously diluted with 1 ml of distilled water. This kit with the blood was incubated for 20 min, in a water bath at 50°C. A half ml of 99mTc solution with 11.1 GBq eluted from a 99Mo/99mTc generator (Instituto de Pesquisas Energéticas e Nucleares, CNEN, São Paulo, Brazil), was added and the incubation continued for another 10 min. Throughout the incubation period the mixture was gently shaken.

The labeled efficiency of RBC with <sup>99m</sup>Tc was determined at the end of this process, as described by Linderkamp et al.<sup>4)</sup> Briefly, after the labeling process and centrifugation, a pellet (red blood cells) and super

natant were isolated. The radioactivity was determined in a well counter NaI (Tl) scintillator. The RBC percentage of radioactivity uptake (%RU) was calculated, dividing the RBC activity by the sum of the RBC plus supernatant activities.

After the labeling process was finished, the mixture was allowed to reach room temperature and then was injected into the same eight dogs, intravennously, at adequate doses.

The same procedure was followed for human volunteers.

Spleen imagings were performed after 20 min of the administration of the labeled and termic denatured RBC. Posterior and left lateral views of the volunteer patients and left lateral views for the dogs were obtained using a 5 inch NaI (TI) rectilinear scanner (Siemens, USA).

## RESULTS AND DISCUSSION

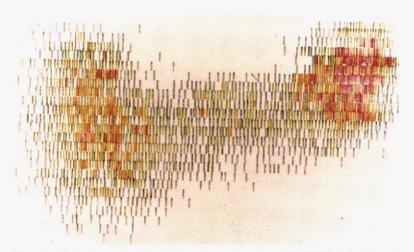
The % RU for the <sup>99m</sup>Tc-labeled and concurrent termic denaturation red blood cells always exceeded 95%.

A protocol to carry out a selective splenic scintigraphy, using <sup>99m</sup>Tc-labeled RBC, obtained with concurrent termic denaturation technique is shown in Table 1.

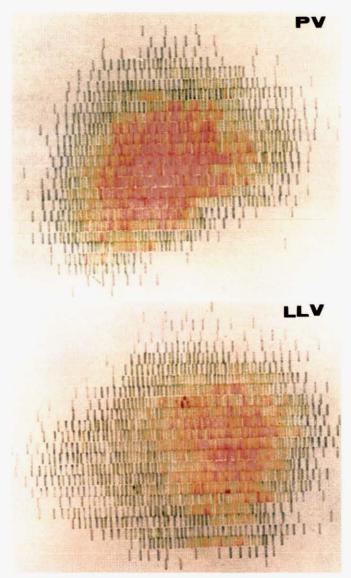
In Fig. 1 a dog spleen scintigraphy is shown. Similar images were obtained for all other dogs studied.

In Fig. 2 is shown the splenic scintigraphic imagings obtained from a volunteer patient. The quality of the other examinations was the same.

No thyroid or stomach uptake was observed in the



**Fig. 1.** Selective splenic scintigraphy of a dog with <sup>99m</sup>Tc-labeled red blood cells using a concurrent termic denaturation technique.



**Fig. 2.** Selective splenic scintigraphy of a human volunteer with <sup>99m</sup> Tc-labeled red blood cells using a concurrent termic denaturation technique. (PV) posterior view and (LLV) left lateral view.

dogs and volunteers. This fact indicates that, when the described technique is employed to label RBC with <sup>99m</sup>Tc, there is no free pertechnetate. The high % RU for the RBC also shows that the radio-activity is significantly bound to red cells.

The analysis of the results obtained with this very simple technique shows its importance in relation to the ones described in the literature. This methodology eliminates several steps, for example, those in the process of centrifugation used to remove the reducing agent or to take out the free <sup>99m</sup>Tc or other substances used such as ethylenediaminetetraacetic acid.<sup>7,17)</sup>

This technique has the advantage of requiring the physical presence of the patient only twice: once when the blood is withdrawn and once when the labeled and termic denatured red blood cells are reinjected. In other methodologies the presence of the patient may be required many more times.<sup>7,14)</sup> The proposed new technique utilizes 1.5 ml of whole blood, a volume much smaller then required in previously described methods.<sup>10,17)</sup>

In spite of the longer time necessary to carry out this new process,<sup>7,10)</sup> we believe that the points discussed above prove the importance and the simplicity of the present methodology.

#### REFERENCES

- Hladik III WB, Saha GB, Study KT: Essentials of Nuclear Medicine Science. Williams and Wilkins, Baltimore, London, 1987, p 90.
- 2) Hegge FN, Hamilton GW, Larson SM, Ritchie JL Richards P: Cardiac chamber imaging: a comparison of red blood cells labelled with <sup>99m</sup>Tc *in vitro* and *in vivo*. *J Nucl Med* **19**: 129-134, 1978.
- 3) Markisz JA, Fromt D, Royal HD, Sacks B, Parker A, Kolodny GM: An evaluation of <sup>99m</sup>Tc-labelled red cell scintigraphy for the detection and localization of gastrointestinal bleeding sites. *Gastroenterology* 83: 394-398, 1982.
- Linderkamp O, Betke K, Fendel H, Klemm J, Lorenzen K, Riegel K: <sup>99m</sup>Tc-labelled red blood cells for the measurement of red cell mass in newborn infants: concise communication. *J Nucl Med* 21: 637-640, 1980.
- 5) Saha GB: Fundamentals of Nuclear Pharmacy. Springer-Verlag, New York, 1984, p 110.
- 6) Subramanian G, Rhodes BA, Cooper JF, Sodd VJ: Radiopharmaceuticals. The Society of Nuclear Medicine, New York, 1975, p 49.
- Sodee DB, Early PJ: Mosby's manual of nuclear medicine procedures. The C V Mosby Company, St. Louis, 1981, p 30.
- 8) Rehanl MM, Sharma SK: Site of <sup>99m</sup>Tc binding to the red blood cell: concise communication. *J Nucl Med* 21: 676–678, 1980.
- Bernardo-Filho M, Moura INS, Boasquevisque EM: <sup>99m</sup>Technetium-labelled red blood cells "in vitro". Arg Biol Tecnol 26: 455-461, 1983.
- 10) Smith TD, Richrds P: A simple kit for the preparation of <sup>99m</sup>Tc-labelled red blood cells. J Nucl Med 17: 126-132, 1976.
- 11) Kempi V, Van der Linden W: Diagnosis of deep vein thrombosis with in vivo <sup>99m</sup>Tc-labelled red blood

- cell. Eur J Nucl Med 6: 5-9, 1983.
- 12) Boasquevisque EM, Antonucci JB, Moura INS, Lopes RLSF, Barboza RG, Canine MS, Bernardo-Filho M: *In vitro* labelling of red blood cells with <sup>99m</sup> Technetium: clinical application for selective splenic scintigraphy. *Radiol Bras.* (in press)
- 13) Boasquevisque EM, Moura INS, Canine MS, Bernardo-Filho M: Lopes RLFS, Antonucci JB: Splenic imaging with <sup>99m</sup>Tc labelled erythrocytes: clinical application. Arg bras Med 60: 117-122, 1986.
- 14) Armas R, Thakur ML, Gottaschalk A: A simple method of spleen imaging with <sup>99m</sup>Tc-labelled erythrocytes. *Radiology* 132: 215-216, 1979.
- 15) Patel MC, Parab PB, Samuel AM, Ganatra RD: Labelling of red blood cell with <sup>99m</sup>Tc after oral administration of SnC12: concise communication. *J Nucl Med* 20: 877-881, 1979.
- 16) Bernardo-Filho M, Canine MS, Lopes RLSF, Boasquevisque EM: Effect of temperature on the "in vitro" labeling red blood cells with Technetium-99m. Ara Biol Tecnol 29: 407-412, 1986.
- 17) Ribeiro-Silva AM, Aragão CE, Marsigo M, Bernardo-Filho M, Mandarim-Lacerda CA: Selective ligadure of lower branch of splenic artery in the dog: Segmentar study. Proceedings of XIII Federative International Congress of Anatomy, Rio de Janeiro, Brazil. p. 66, 1989. (abstract)
- 18) Gutfilen B, Souza JEQ, Maciel OS, Martinho MJ, Gabbay S, Boasquevisque EM, Bernardo-Filho M: A simple kit to label red blood cells with Technetium-99m. Proceedings of Fourth European Symposium on Radiopharmacy and Radiopharmaceuticals, Baden, Swiss. p. 83-84, 1991 (abstract)
- 19) Bernardo-Filho M, Gutfilen B, Souza JEO, Maciel OS, Boasquevisque EM, Gabbay S, Martinho MJR, Hasson-Voloch A: Labeling of red blood cells with 99mTechnetium: a very simple kit. *Acta Med Biol* 1992. (in press)