Failure to label red blood cells adequately in daily practice using an in vivo method: methodological and clinical considerations

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Received 5 July and in revised form 10 September 1994

Abstract. This study was conducted to evaluate the frequency and possible causes of poor red blood cell (RBC) labelling when performing equilibrium gated blood pool (GBP) radionuclide angiography at rest with an in vivo method. The influence of the mode of administration on tagging efficiency was studied by investigating the image quality in 160 patients referred for evaluation of left ventricular (LV) function prior to or after coronary angiography, while using a roughly standardized administration protocol. The patients were subclassified into four groups according to the way both molecules involved in the tagging procedure were administered. When poor image quality was found (in 9.4% of the patients), the labelling efficiency was quantified and the frequency of failed tagging in each group was calculated. A significant association was found between poor labelling and the use of a Teflon catheter or butterfly needle for the injection of the stannous agent. In another 737 patients, in order to avoid the problems observed in the first group, a strict administration protocol was applied to analyse the frequency of poor tagging and its possible causes. Suboptimal image quality was present in 88 patients (11.9%). Quantitatively confirmed poor tagging was present in 36 of the 88 (40.9%, or 4.9% of the whole group); the remaining 52 patients showed borderline normal labelling (>80% bound fraction). Drug interference was studied by comparing the medications used by the 36 patients showing poor binding with those used by 44 control patients. A significant relationship was found between the use of heparin or chemotherapy and the tagging. The influence of several clinical factors on the labelling was also investigated. A significant correlation was found between advanced age, particularly when associated with acute severe disease, and poor labelling efficiency. Finally, in 36 patients with poor labelling, a second GBP test was performed, using either the modified in vivo method or a new commercially available kit for in vitro tagging. This allowed us to evaluate the accuracy of the obtained ejection fraction value when a suboptimal image set is used, and to assess the feasibility of using the new kit in daily practice.

Key words: Equilibrium gated blood pool radionuclide angiography – In vivo method – Poor labelling


Introduction

Equilibrium gated blood pool (GBP) radionuclide angiography at rest is a worldwide-accepted non-invasive and accurate parameter for the determination of ventricular function, whose prognostic value in patients with coronary artery disease has recently been re-emphasized [1, 2].

Although overall agreement exists that in vitro labelling is the best method for GBP studies, many centres prefer to use in vivo tagging because of its ease of use and the reduction in time consumption. Since the description of the method for in vivo red blood cell (RBC) labelling by Pavel et al. in 1977 [3], it has been widely applied all over the world, using different stannous compounds for the tinning procedure, with reported labelling values from 60% to 90% [4].

The mechanism involved in the RBC labelling process remains uncertain. Initial studies postulated the following schema: Stannous ion diffuses freely into the cell and, due to intracellular binding, remains intracellular. Pertechnetate also diffuses freely in and out of the cell, but in the presence of Sn²⁺ is reduced and binds mainly to the beta chain of the globin within a haemoglobin [4]. In some cases, a part of the injected tin reduces technetium-99m extracellularly, resulting in a lower binding to the RBC and thereby in reduced labelling. More recent-
ly, however, Seldin et al. suggested that the majority of RBC-bound $^{99m}$Tc (up to 80%) is not associated with haemoglobin but remains in a more labile intracellular pool, either intracytoplasmic or on the cell membrane itself, as proven by the drastic reduction of $^{99m}$Tc RBC activity after treatment of the RBC with neuraminidase, which is known to cleave the terminal sialic acid group on the erythrocyte membrane glycoprotein, with loss of the surface negative charge [5].

In our daily practice of cardiac nuclear medicine, we observed that in some patients who underwent an equilibrium GBP study using the in vivo method, the image quality was too poor to permit adequate interpretation. We decided to study the frequency and possible causes of such poor labelling.

**Material and methods**

Between October 1993 and June 1994, 897 patients referred for a GBP equilibrium rest study (prior to or after coronary angiography (545 patients)); iterative controls in patients receiving cardiotoxic drugs (128 patients); left ventricular (LV) function evaluation in an elderly population (224 patients) were studied using an in vivo method.

First, 160 patients (37 women and 123 men; mean age ±SD: 55.4±7.1 years), all referred for evaluation of LV function within 24 h of coronary angiography, were injected with stannous medianate (Amerscan stannous agent, Amersham, England, 4.0 mg/vial diluted in 6 ml isotonic saline), using either a standard dose (1.3 mg/patient) or a dose related to body weight (15 μg/kg), followed after a timing period of 20-30 min by 740 MBq $^{99m}$Tc in 0.4-0.7 ml isotonic saline (only eluate of less than 4 h was used). Both injections were performed using a needle, a butterfly needle or an intravenous cannula.

Subsequently, due to the very obvious influence of some methodological parameters on the labelling efficiency, we decided to apply a strict standardized administration protocol to study a further group of 737 patients. The dose of stannous agent was adapted to the patient's weight (15 μg/kg), the interval between the two injections was 30 min precisely, freshly eluted $^{99m}$Tc (740 MBq) was diluted to 1.5 ml by adding physiological saline prior to the administration, and the two injections were performed in different veins using metal needles (for both injections if possible, but at least for the administration of the stannous agent). A separate group of 44 patients (nine women and 35 men; mean age ±SD: 63.1±10.7 years) referred for similar reasons and showing good image quality was used as a reference population.

Acquisition was performed 10 min later on a short field of view gamma camera, 20 cm detector size (Apex 215M, Elscint, Israel), equipped with a low-energy, all-purpose collimator (22 MSC-3), using a 64x64 matrix and 32 bins/heart cycle. A total of 6.4 million counts was obtained for both LAO and LPO views.

Processing consisted in drawing a single diastolic region of interest (ROI) (semi-automatic program), unless the image quality was so poor that a full manual two-ROI program had to be used.

The image quality was first qualitatively and semiquantitatively assessed, taking into account the activity in the heart compared to that in the salivary, gastric and thyroid tissues, and the measured target to background ratio (TBR). Qualitatively, the image sets were considered good when a clear delineation was observed between the end-diastolic (ED) and end-systolic (ES) edges, suboptimal when the edges were more difficult to define but still could be drawn without modifying the baseline and the brightness of the computer, and poor when the LV ED and ES limits were almost invisible.

The TBR was calculated as follows by using two square ROIs (eight pixel size) placed in the centre of the LV and in the surrounding tissues:

\[ \text{TBR} = \frac{\text{LV activity (counts)}}{\text{surrounding activity (counts)}}. \]

A TBR value >2.7 was considered high (good quality), between 1.3 and 2.7 intermediate (mild quality) and <1.3 low (poor quality), referring to the TBR reported by Strauss et al. [6].

If suboptimal or poor image quality was considered present, the labelling efficiency (or bound fraction) was calculated using a single blood sample taken 20 min after the injection of $^{99m}$Tc, and according to the following formula:

\[ \text{Labelling efficiency (\%)} = \frac{\text{activity in RBC fraction (cpm) after centrifugation and washing}}{\text{activity in whole blood (cpm)}} \times 100. \]

For this purpose, a 2 ml blood sample on EDTA was withdrawn, from which 50 μl whole blood was diluted in 1 ml saline solution. A 50-μl sample of the diluted blood was used to measure whole blood activity. RBC activity was measured by washing another 50-μl sample 3 times (dilution with 2 ml saline solution, followed by centrifugation at 3500 g/min for 5 min using an EBA 3S centrifuge (Hettich, Germany)). All samples were counted during 60 s in a well counter (LKB 260 Multigamma II, Wallac Oy, Finland). In order to exclude technical errors, the washing and counting procedure was performed twice.

As Srivastava and Chervu [4] described labelling efficiencies ranging from 60% to 90%, and Strauss et al. [6], from 75% to 85%, we decided to take a 65% binding as the lowest threshold. A bound fraction of more than 90% was judged high, between 65% and 90% borderline normal, and less than 65% low.

Thirty-six of the patients showing poor labelling were submitted to a new GBP test: a new commercially available cold kit for in vitro labelling without the need for centrifugation (Ultratag RBC, Mallinckrodt, The Netherlands) was used in 25 of them, and the modified in vivo method in the 11 others. This method was applied as follows: an intravenous injection of 15 μg/kg body weight of stannous medronate was followed after an interval of 30 min by withdrawal of a 3-ml blood sample, using a syringe containing 15 U heparin and 740 MBq freshly eluted $^{99m}$Tc in 1.5 ml physiological saline. After an incubation time of 10 min in vitro, the entire contents of the syringe was re-injected to the patient using a metal needle.

Technical parameters as well as clinical considerations were studied in order to detect possible causes of poor labelling efficiency. The influence of medications was examined by comparing the 36 patients with poor labelling with the reference population showing good image quality and a high bound fraction.

Statistical analysis was performed using Fisher's exact test and the Pearson chi-square test (exact probabilities calculated with StatXactR, Cytel Software Corporation, UK). A $P$ value of 0.05 or less was considered significant.