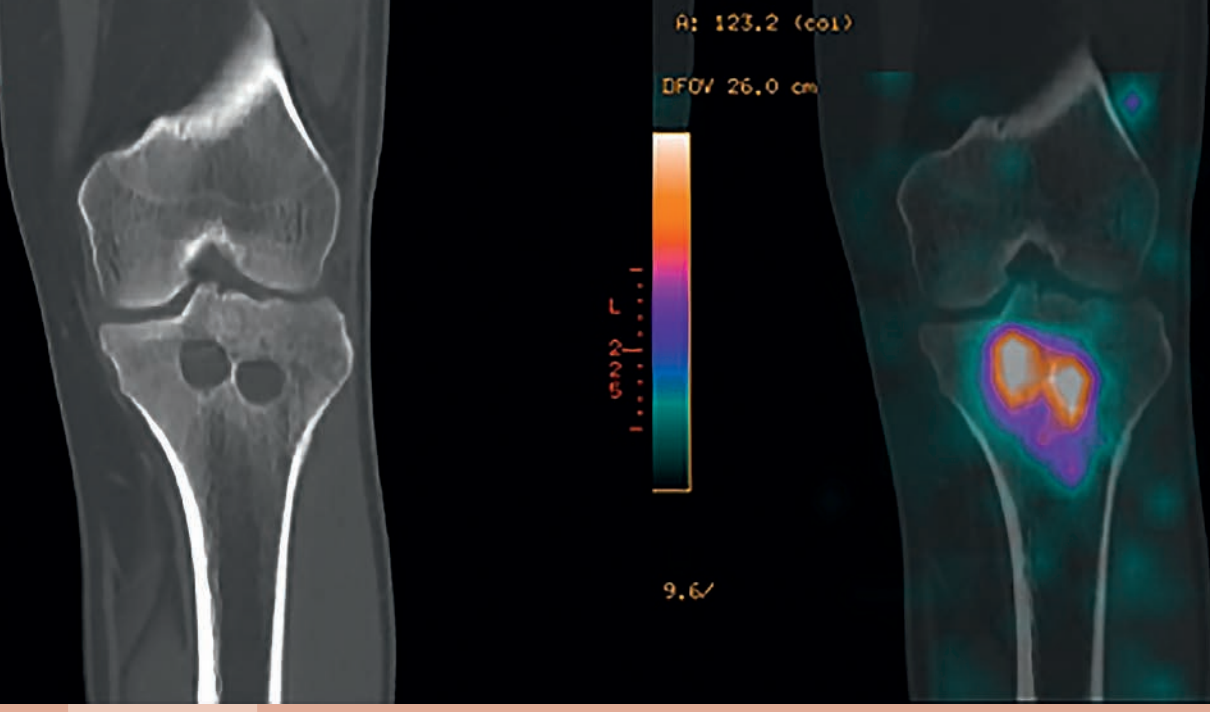

IAEA HUMAN HEALTH SERIES

No. 5

Radiolabelled Autologous Cells: Methods and Standardization for Clinical Use

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RADIOLABELLED AUTOLOGOUS CELLS: METHODS AND STANDARDIZATION FOR CLINICAL USE

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FOREWORD

The clinical use of radiolabelled autologous blood cells is considered of value for medical research and the diagnosis of disease. However, the experience of nuclear medicine physicians and radiopharmacists has shown that the labelling procedure is time consuming, relatively expensive and exposes the operator and patient to several risks. Standardizing radiolabelling methods for autologous blood cells can help to provide international standards for quality assurance and control and to summarize indications, norms and ethical considerations in the clinical use of radiolabelled cells. This use includes the in vivo or ex vivo labelling with radioactive isotopes of white blood cells (granulocytes or mixed leukocytes, monocytes and lymphocytes), red blood cells and platelets. This publication provides a framework for the standardization of the methods using radiolabelled autologous blood cells, including dendritic cell labelling and stem cell labelling for their particular importance in medical research and for their great potential for clinical applications.

In order to standardize the labelling procedure, imaging modality and clinical applications of radiolabelled white blood cells, several nuclear medicine societies and other institutions have been involved in preparing practical and procedural guidelines as well as evidence based indications for the correct application of labelling techniques. The national guidelines and international society guidelines on this topic have small differences in protocols and performance of quality control, depending on local legislation. This publication aims at becoming an important reference and milestone for the worldwide standardization of radiolabelling procedures, quality control, safety procedures, clinical indications, image acquisition and image interpretation in the field of autologous product labelling for diagnostic purposes. It is therefore hoped that those involved in the fields of nuclear medicine, pharmacy and radiopharmacy will use this publication as a reference for national revision and updating of their protocols. This publication is intended as a guide to help physicians, particularly nuclear medicine physicians in training or already with experience in cell labelling procedures, biologists, radiopharmacists and laboratory technicians to initiate and maintain the availability of labelled autologous products. The aim is to provide the necessary requirements and information to perform cell labelling and subsequent imaging in agreement with established guidelines and norms from international pharmacopoeia and legislation.

This publication has been prepared by a panel of international experts, including members of the International Society of Radiolabeled Blood Elements together with members of the Inflammation/Infection Task Group of the Italian

Association of Nuclear Medicine and of the European Association of Nuclear Medicine and has been revised by other international experts. The IAEA gratefully acknowledges the inputs of the contributors to this publication as well as the reviewers.

The IAEA officers responsible for this publication were K.K. Solanki and M. Dondi of the Division of Human Health.

CONTENTS

1. INTRODUCTION

1.1. BACKGROUND

The clinical use of radiolabelled autologous blood cells is considered of value for the diagnosis and follow-up of different diseases, such as osteomyelitis, fevers of unknown origin (FUO) and infected vascular prosthesis, and for research purposes. Although ^{67}Ga-citrate is a widely distributed and extensively used radiopharmaceutical, in particular in the field of infection imaging, radiolabelled white blood cells are still the gold standard technique for infection detection [1.1–1.4]. However, the experience of nuclear medicine physicians and radiopharmacists has shown that the labelling procedure is time consuming, relatively expensive and exposes the operator and patient to several risks. The different strategies which have been developed to bypass these practical problems include:

— Monoclonal antibodies against leukocyte antigens;
— Polyclonal human immunoglobulin G, which can also be used to study inflammatory diseases;
— Radiolabelled albumin microcolloids.

Other products are under development, such as radiolabelled cytokines and radiolabelled antibiotics, that may be relevant in the treatment of patients with infection. In addition, ^{18}F-fluorodeoxyglucose (FDG) and positron emission tomography in connection with X ray computed tomography (PET/CT) is a unique method for the diagnosis of inflammatory and infectious processes [1.5]. Still, the body of evidence accumulated so far regarding the use of radiolabelled white blood cells makes their use the method of choice [1.6]. In fact, radiolabelled blood cells have become a successful milestone in the last 30 years of nuclear medicine, since it was first demonstrated that ^{111}In-oxine can be used as a radiopharmaceutical to radiolabel white blood cells and ^{99m}Tc as a label for red blood cells. Since then, there has been phenomenal growth in the use of these techniques to satisfy clinical demands.

Red blood cell labelling is also frequently performed. The first report of such labelling was published in 1967 [1.7]. The procedure is established for a wide range of clinical applications and conducted regularly in most nuclear medicine departments. In vivo, in vitro and combined in vivo/in vitro (in vivitro) techniques are available to label red blood cells. Optimal labelling of the blood pool is essential for a diagnostic study. In vitro red blood cell labelling can be performed in a closed system, and there is less handling of the blood than with

the labelling of, for example, white blood cells. The hazard to staff and accidental contamination with the hepatitus C virus or the human immunodeficiency virus (HIV) is low. It is, however, always important to be aware of some precautions when labelling any blood element, since the final, injectable product cannot be sterilized.

1.2. OBJECTIVE AND SCOPE

In order to standardize the labelling procedure, imaging modality and clinical applications of radiolabelled white blood cells, several nuclear medicine societies and other institutions have been involved in preparing practical and procedural guidelines as well as evidence based indications for the correct application of labelling techniques.

This publication provides a framework for the standardization of the methods using radiolabelled autologous blood elements (namely, white blood cells, red blood cells and platelets). The procedures for labelling other cell types, such as lymphocytes, monocytes, stem cells and dendritic cells, are also considered, although these techniques are still confined to research protocols in most countries.

This publication is intended as a guide to help nuclear medicine physicians, radiopharmacists and laboratory technicians to initiate and maintain the availability of labelled autologous products.[1] The aim is to provide the necessary requirements and information to perform cell labelling and subsequent imaging in agreement with established guidelines and norms from international pharmacopoeia and legislation. Appendix III and Refs [1.8–1.13] provide examples of recognized methodological protocols for cell labelling and protocols for image acquisition and interpretation.

1.3. USE OF LABELLED CELLS IN CLINICAL PRACTICE

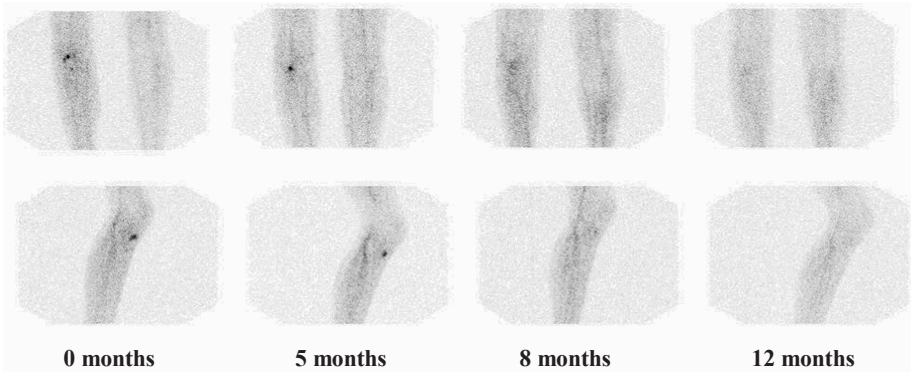
1.3.1. White blood cells

White blood cells were first labelled in 1976 with ^{111}In-oxine [1.14], and their applications were first discussed during a symposium on radiolabelled

[1] Guidance provided here in the form of ‘should’ statements, or simply in the present tense indicative, describing good practices, represents expert opinion but does not constitute international consensus recommendations on how to meet the relevant requirements.

cellular blood elements in November 1979 in New York. This generated a tremendous impetus among researchers throughout the world in a variety of disciplines. By early 1980, there were as many as 600 Investigational New Drug (IND) applications filed with the United States Food and Drug Administration (FDA). In 1985, ^{111}In-oxine was approved by the FDA as a low risk substance. Since then, the technique of using radiolabelled white blood cells — including the use of ^{99m}Tc-hexamethylpropyleneamine oxime (^{99m}Tc-HMPAO) or ^{99m}Tc-stannous fluoride colloids (^{99m}Tc-SnF_2) — rapidly spread over the world for many different clinical applications, including (see Fig. 1.1):

— Osteitis and osteomyelitis;
— Detection of infections related to implants, prostheses and vascular grafts;
— Inflammatory bowel diseases (IBDs) and appendicitis;
— Study of patients with FUO;
— Diagnosis of abscesses and soft tissue infections;
— Follow-up of patients with infective diabetic complications;
— Diagnosis of other microbial infections.

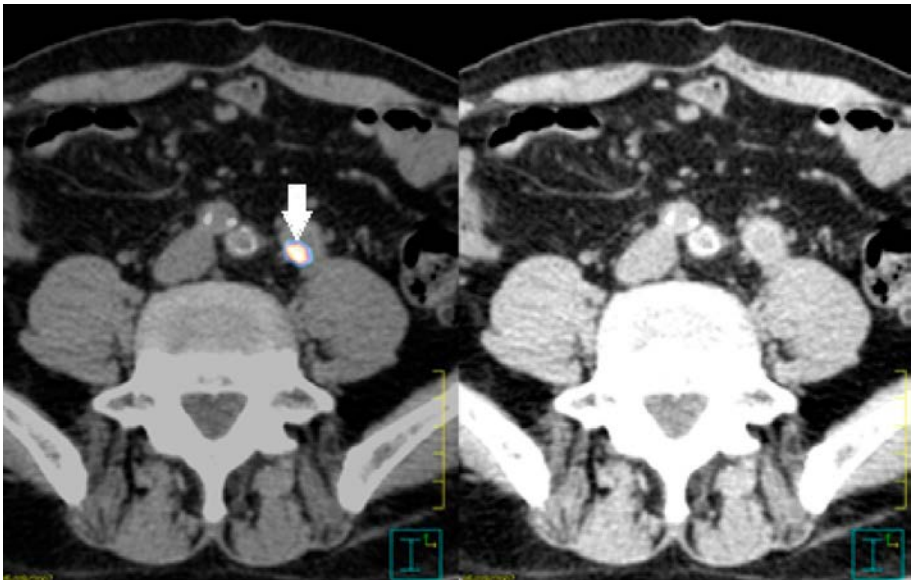


Note: The ^{99m}Tc-HMPAO labelled leukocyte study demonstrates a reduced uptake in time (at diagnosis and after 5, 8 and 12 months) corresponding to the efficacy of the antibiotic treatment (upper row: anterior images, lower row: lateral images).

FIG. 1.1. A 37 year old women with an infected fracture of the upper right tibia.

White blood cells have also been labelled with FDG (see Fig. 1.2). However, despite encouraging initial results and high quality images, the clinical use has been limited because acquiring images after 2–4 h is not possible.

In the 1980s, lymphocytes labelled with ^{111}In-oxine were also used for the diagnosis of autoimmune diseases, chronic graft rejections, chronic infections



Note: Images show the accumulation of radiolabelled leukocytes within the graft 1 h after administering labelled cells.

FIG. 1.2. PET/CT imaging of an infected vascular graft using FDG labelled autologous white blood cells (courtesy of A. Signore, Sapienza University of Rome).

and other chronic inflammatory disorders. However, the high radiosensitivity of these cells and the demonstration that both ^{111}In and its chelators — oxine (8-hydroxyquinoline) and tropolone — may impair cell function have severely limited further clinical use of this technique [1.15, 1.16].

The European Association of Nuclear Medicine (EANM) has recently published revised guidelines for white blood cell labelling with ^{99m}Tc-HMPAO and ^{111}In-oxine. These procedures will be referred to in this publication [1.10, 1.11].

1.3.2. Red blood cells

Labelling red blood cells is widely used in clinical nuclear medicine for several imaging applications:

— Imaging of the cardiovascular system, particularly wall motion studies using multiple gated acquisition (MUGA) scans;
— Detection of gastrointestinal bleeding;
— Detection of hepatic haemangiomas;

— Spleen imaging with heat damaged cells;
— Red blood cell volume determination (to differentiate between absolute and relative polycythaemia);
— Red blood cell sequestration and survival studies.

In contrast to labelling white blood cells and platelets, it is relatively easy to label red blood cells. In addition, the in vitro labelling technique is not as time consuming as that of labelling other blood cells. Over the last 30 years, however, red blood cells have more often been labelled in vivo than in vitro because it is an easier procedure. However, it needs to be kept in mind that there is less control over the labelling efficiency when the in vivo technique is performed [1.17–1.23].

1.3.3. Platelets

The radiolabelling of platelets was originally performed with ^{51}Cr to monitor cellular kinetics. Thakur et al. were the first to introduce ^{111}In-oxine, which enabled the visualization of abnormal platelet deposition in vivo [1.24]. Radiolabelling platelets is still the method of choice to assess survival. It can also be used to image abnormal (arterial and venous) vascular or thrombotic lesions [1.25]. The use of PET tracers [1.26], such as ^{68}Ga-oxine, has not been a success since platelets need hours to accumulate at pathological sites, both in the arterial and the venous circulation, which is not feasible with the short half-life of most positron emitting isotopes.

Other complexes, such as oxine sulphate, tropolone and mercaptopyridine N-oxide (MPO), have not turned out to be better. Technetium-99m (in a complex with HMPAO and oxine, among others) does not allow concomitant lifespan calculation, although sensitivity and specificity for imaging are reported to be comparable. Platelets labelled with ^{99m}Tc-HMPAO show a higher labelling efficiency when performed in saline (approximately 50%) compared with plasma (40%). Overall, however, the labelling efficiency is much lower when compared with ^{111}In complexes. A longer incubation time of more than 10 min (30 min is better) is required. Owing to the absence of proteins and calcium, the incubation of platelets in acid citrate dextrose (ACD) saline increases the membrane permeability and subsequently enhances the influx of ^{99m}Tc-HMPAO. Labelling efficiency is independent of the HMPAO concentration. No significant differences between l-HMPAO and d-HMPAO have been found. Oxine, tropolone and MPO have been used for the ^{68}Ga labelling of pig, dog and human platelets. With ^{68}Ga-oxine, however, around 50% of the tracer was removed by the first wash. Gallium-68-MPO resulted in a somewhat lower labelling efficiency, but the loss of tracer was again much higher (10–20%) than ^{111}In (<1%). A limiting factor was also the potential radiation dose to the spleen. The labelling time

of 1 min only at 37°C already results in a labelling efficiency of around 90%. For safety reasons, however, an incubation time of 5 min is recommended.

The half-life of PET tracers is generally too short to enable the monitoring of abnormal platelet residence, accumulation or thrombosis. The positron labelled platelets reflect more a circulating population of cells, eventually including acute trapping, while ^{111}In labelled platelets seem better to image residing or sequestrated cells.

1.4. OTHER RADIOLABELLED AUTOLOGOUS CELLS

1.4.1. Lymphocytes

Lavender et al. were the first to label autologous lymphocytes and subsequently to monitor the kinetics in normal subjects and in patients with Hodgkin's disease [1.27]. Lymphocytes show a high radiation sensitivity and consecutively, especially at activities higher than 185 kBq/10^8 cells, a limited viability — particularly after 72 h and more [1.28]. As cytogenetic studies had revealed that labelling might not be harmless, the application was stopped [1.15, 1.16]. Lymphocyte labelling allowed the monitoring of homing in patients with autoimmune diseases [1.29] and lymphatic malignancies [1.28], mainly in Hodgkin's disease, while low malignant lymphoma and chronic lymphatic leukaemia mostly showed negative imaging results. These data have not been examined or validated further, owing to concerns related to exposure to radiation of sensitive cells such as lymphocytes.

1.4.2. Monocytes

Heyns et al. were the first to show the possibility of isolating a sufficient number of monocytes from non-human primate blood [1.30]. The attempt consistently to image atherosclerotic lesions failed, as the number of cells isolated was limited, contamination with other cells could not be sufficiently avoided and a low target to background ratio was achieved by the few monocytes deposited in the vascular lesions. Monocyte kinetics showed a transient image of the lungs to a varying extent within the first 15 min after reinjection and a predominant spleen uptake. Activity in the blood rapidly disappeared, with a redistribution peak occurring somewhere between 2 and 8 h. Although radiolabelled low density lipoprotein (LDL) and monocytes co-localized, information on sensitivity and specificity for the extent or activity of vascular lesions has never been obtained owing to the methodological problems mentioned above. The viability

is acceptable up to 370 kBq/10^8 mononuclear cells, and it shows a significant decrease at higher activities and, in particular, after 72 h.

1.4.3. Stem cells

Stem cells are currently regarded as magic seeds with the potential to generate organs and to cure diseases. When radiolabelled, labelling efficiencies in the range of 25–60% have been reported [1.31–1.33]. However, no specific information concerning labelling characteristics of the various types of stem cell is available. Functional parameters to be examined include capillary tube formation, surface markers (CD73 and C90) and microscopic monitoring. Extensive studies of cellular viability of mesenchymal stem cells using ^{111}In-tropolone do not report any adverse effects [1.31]. Bone marrow derived stem cells, bone marrow derived mononuclear cells (subgroup: endothelial progenitor cells) and also mesenchymal stem cells, or adipose tissue derived cells, have all been used. Cytogenetic studies are not available. After reinjection, temporary lung uptake can be observed. There is virtually no information available regarding viability, proliferation and radiation damage. Unsolved key questions include further target cells, homing and organ specific differentiation, and safety issues.

1.4.4. Dendritic cells

Dendritic cells form an individualized pharmaceutical that is increasingly used in oncologic immunotherapy. Immunization via ex vivo generated dendritic cells has recently become of key interest for various clinical applications. Optimal site of injection, migration patterns, qualitative and quantitative distribution, pharmacokinetics and survival still remain to be elucidated.

Dendritic cell migration has been shown to be reduced in hyperlipidemia, restored after high density lipoprotein (HDL) addition and increased by statin treatment [1.34, 1.35]. Whether the labelling conditions are also affected by LDL cholesterol is so far unknown. On the other hand, Quillien et al. report that mature dendritic cells more commonly migrate towards nodes and that their migration tends to increase up to 48 h after injection [1.36]. They never observed migration to more than one node. Blocklet et al. report that more than half of the radioactivity is released between 16 and 21 h after ^{99m}Tc-HMPAO labelling [1.37]. They did not witness any migration with immature dendritic cells.

Laverman et al. aimed to successfully develop ^{111}In radiolabelled tumour derived peptides after conjugation with diethylenetriaminepentaacetic acid (DTPA) [1.38]. Much less data are available about the in vivo fate of the major histocompatibility complex (MHC). Laverman et al. show that binding to MHC is temperature dependent: the highest at 4°C; the lowest at 37°C. Apparently,

there is no significant influence of incubation time and temperature. Therefore, incubation for 10 min at 22°C is both practical and recommendable.

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2. GENERAL REQUIREMENTS FOR RADIOLABELLING AUTOLOGOUS CELLS

The processes followed for labelling autologous products are not licensed. However, they should comply with good clinical standards. Most professional associations in IAEA Member States have locally accepted regulations which prescribe a certain level of quality control and assurance for the labelling of autologous products. This section summarizes the known standards in an attempt to make them accessible to those working in the field of nuclear medicine.

2.1. REGULATORY REQUIREMENTS

Components used for the radiolabelling of autologous products should be manufactured in accordance with the basic principle of good manufacturing practice (GMP) and with national regulations on the production of medicines and any other regulations on a comparable level.

2.1.1. Requirements for licensed products

Licensed products and procedures should be used or performed according to the instructions of the manufacturer. Note that for liability reasons, most procedures for radiolabelling autologous materials are not licensed by the manufacturer of the products used in these procedures.

2.1.2. Requirements for licensed products applied to non-approved indications

In general, most professional organizations support the use of licensed products for non-approved indications when there is substantial evidence in the literature supporting its unlicensed use. A general requirement for the unlicensed use of licensed materials is to obtain informed consent from the patient, followed by registration in the patient file. The informed consent is more than a patient signed consent form: it is a process of communication between a patient and his or her physician that results in the patient's authorization, or agreement, to undergo a specific medical intervention. In the communication process, the

physician providing or performing the treatment or procedure (not a delegated representative) should disclose and discuss the following points with the patient:

— Patient's diagnosis, if known;
— Nature and purpose of a proposed treatment or procedure;
— Risks and benefits of a proposed treatment or procedure;
— Alternatives (regardless of their costs or the extent to which the treatment options are covered by health insurance);
— Risks and benefits of the alternative treatment or procedure;
— Risks and benefits of not receiving or undergoing a treatment or procedure.

In turn, a patient should have the opportunity to ask questions to elicit a better understanding of the treatment or procedure, so that he or she can make an informed decision to proceed or to refuse a particular course of medical intervention. This communication process is both an ethical obligation and a legal requirement defined in the statutes and case law of most States.

2.1.3. Compounding non-licensed products

Compounding includes:

— Formulation of radiopharmaceutical reagent kits from raw ingredients for radiopharmaceutical preparation;
— Addition of reagents to commercial kits to modify or enhance the performance of radiopharmaceuticals (shelf life extension and fractionation);
— Synthesis from raw materials.

Compounding should follow recognized pharmacopoeia protocols whenever available; approval by an institutional committee is otherwise required. The process of compounding radiopharmaceuticals needs to be under the supervision and responsibility of a recognized nuclear physician or suitably qualified professional, ideally a radiopharmacist. Compounding is limited to clinical practice according to a medical doctor's prescription or requisition for a specific patient. Patent protected radiopharmaceuticals should not be compounded. When, however, patented reagent kits cannot be readily obtained from a commercial source, limited compounding should be performed to meet the urgent medical needs of an identified individual patient. In this case, the prescriber needs to be informed that a reagent kit will be compounded to replace the commercial product. Compounded radiopharmaceuticals are not for sale and are not to be advertised.

2.2. AVAILABLE GUIDELINES

For most radiopharmaceuticals used in the protocols for cell labelling, pharmacopoeia monographs are available in several countries or at a higher level (e.g. continental). Operational Guidance on Hospital Radiopharmacy: A Safe and Effective Approach [2.1] provides an overview of regular standards that should be followed to comply with good radiopharmacy practice. For some available guidelines on this subject, see Refs [2.2–2.7].

2.3. ETHICAL ISSUES

2.3.1. Medical issues

If applicable, informed consent for white blood cell scintigraphy needs to be signed by the patient or legal guardians, depending on local regulations. However, this is not necessary in Member States of the European Union. Nevertheless, the patient's condition should be such that he or she can undergo the blood labelling and scan. This needs to be carefully evaluated by the referring medical doctor and nuclear medicine physician, possibly in a preliminary visit before booking the scan.

2.3.2. Radioactivity issues

Personnel should be monitored by approved personal dosimeters. Readouts of the dosimeters should be regularly checked and recorded using appropriate loggings. These regular checks may be supplemented by electronic dosimeters. A frequent monitoring system for both personnel and working areas needs to be implemented to follow the performance of cell labelling and related activities. When possible, the use of ^{99m}Tc for the labelling of cells is preferred over ^{111}In or ^{51}Cr, as the radiation dose to the patient and operator is regularly lower [2.8].

2.3.3. Protection of patients

Working with patient blood has legal, ethical and health implications. It therefore requires stringent procedures to avoid contamination of patient material. Personnel should be sufficiently trained for this purpose, and the training should be preferably certified by an authorized organization. Class A laminar flow hoods should be used in restricted areas, according to local rules or recommendations. Since administering incorrect blood material to a patient may be extremely dangerous, this needs to be prevented by strict standard operating procedures

(SOPs). The rule 'one patient's blood per procedure' is an effective basic approach used in many centres to prevent incorrectly administered labelled cell material. Labelling white blood cells of different patients should be carried out at physically separated locations. At all times, correct identification of patients' blood products should be guaranteed. All syringes, tubes and any material in contact with the patient's blood components should be clearly labelled with the patient's name and a barcode or colour code.

2.3.4. Pyrogens

When testing bacterial endotoxins (pyrogens), the limulus amoebocyte lysate (LAL) test may be useful. The test needs to be applied to all liquid reagents that are aliquoted from stock solutions. The test may also provide information on aseptic conditions of the procedure and can be used as a lead procedure for the optimization of validation.

2.3.5. Microbial contamination

The preparation of labelled cells or blood cell elements needs to be done aseptically. Primarily, this means that training in aseptic techniques needs to take place before the personnel are qualified to do the radiolabelling. Furthermore, dedicated clothing, sterile gloves, sterile syringes and needles should be used for each preparation. To warrant these conditions, preparation needs to be done in a down flow laminar flow hood (ideally a safety bench class IIa). There should be a sanitation programme for the laminar flow hood and its surrounding room, as well as the microbiological monitoring of the preparation area.

2.3.6. Final safety considerations

To assure patient and personnel protection from bacterial or viral contamination, and having overviewed most local rules and recommendations, the panel of experts which prepared this publication recommends that:

(1) Personnel should follow a specific training program certified by national or international societies. Such training should be updated every five years.
(2) Labelling procedures should be prepared using a down flow laminar flow hood class IIb positioned in a class C environment or using an isolator in a class D environment.
(3) Only one patient's blood should be manipulated in a class A laminar flow hood at a time. Multiple patients' samples can be labelled in physically separated laminar flow hoods, although in the same room.

(4) If closed sterile disposable systems are used, a laminar flow hood in a class D environment is sufficient.
(5) Sterile gloves and sterile disposable materials need to be used.
(6) Sterile, GMP produced reagents should be used. If individual doses are dispensed from a stock solution, a random pyrogens test needs to be performed for validation.
(7) Validation of the whole procedure and required personnel always needs to be conducted at the beginning by performing three consecutive separation and labelling procedures with all quality controls:
   (i) Labelling efficiency;
   (ii) Visual examination;
   (iii) Sterility;
   (iv) Viability;
   (v) Cell recovery;
   (vi) Release test;
   (vii) Pyrogen test.
(8) Revalidation of the process should be performed once every six months by performing only one procedure with all quality controls. A change in the protocol, equipment or personnel requires a new validation in triplicate.

2.4. PERSONNEL

2.4.1. Specific training for personnel

Basic skills to train personnel include:

— Teaching the use of equipment and its maintenance;
— Teaching local rules and recommendations;
— Teaching how to work in aseptic conditions;
— Use of a class IIa safety cabinets;
— General information on radioprotection and use of isotopes;
— Practical skills to label cells and blood elements;
— Practical skills to perform required quality controls;
— Record keeping requirements;
— Basic information on image acquisition, interpretation and in vivo quality controls;
— Study of available guidelines and pharmacopoeia.

Several societies, including the International Society of Radiolabeled Blood Elements (ISORBE), EANM and national societies, organize courses for

training personnel in cell labelling. ISORBE proposes the following training programme, which may be adopted by other societies, to standardize training and preparation of personnel. The total time needed for the diploma is 36 h (25 h theory, 11 h practical).

(1) Basic introduction to dosimetry and radioprotection (2 h theory).
(2) Basic introduction to microbiology and infectious diseases (2 h theory).
(3) Basic introduction to immunology and inflammation/infection (2 h theory).
(4) Basic introduction to laboratory techniques and biochemistry (2 h theory, 1 h practical).
(5) How to deal with possible infective patients (1 h theory).
(6) How to work in sterile and aseptic conditions (1 h theory, 1 h practical).
(7) How to work in GMP conditions (2 h theory, 1 h practical).
(8) How to work with SOPs (1 h theory).
(9) How to perform quality controls (2 h theory, 2 h practical).
(10) How to purify and label autologous leukocytes, erythrocytes and platelets in isolator and using sterile disposable closed devices (2 h theory, 6 h practical).
(11) How to deal with and to report adverse reactions (1 h theory).
(12) How to acquire, elaborate and interpret images (2 h theory).
(13) Knowledge of common pitfalls and artefacts (2 h theory).
(14) Knowledge of clinical indications for the use of radiolabelled autologous cells (3 h theory).

2.4.2. Personnel safety considerations

2.4.2.1. Radiation risk

It is necessary to protect both patients and staff from unwarranted radiation exposure. The risks associated with radiation exposure in nuclear medicine are generally negligible in most cases. The two leading principles in nuclear medicine, as in all medical practice, are justification and optimization: any nuclear medicine procedure needs to be justified by an appropriate request; and the procedure needs to be optimized to obtain the best possible results. Staff should be familiar with the ALARA (as low as reasonably achievable) principle to reduce the exposure to radiation to the minimum level compatible with successful clinical use. For the implementation of these principles, a thorough programme of quality assessment and quality control needs to be practised in all aspects of nuclear medicine [2.1, 2.9].

2.4.2.2. External radiation monitoring

Procedures for monitoring external radiation include:

(a) Areas and neighbouring areas in which radiation sources are used or stored should be monitored at regular intervals.
(b) Radiation should be checked before using radiation sources, after any significant modification of set-up and also periodically during operation.
(c) Frequency of radiation checking should be determined by the area radiation protection officer depending on the nature and scale of operations carried out in the area.
(d) All radiation checking should be carried out and a proper record should be maintained by the radiation protection personnel.

2.4.2.3. Surface contamination monitoring

The principal objectives of monitoring surface contamination can be summarized as follows:

— Detect failure of containment;
— Detect shifts from good operating procedures;
— Limit surface contamination to levels at which the general standards of laboratory housekeeping are adequate;
— Provide information for the planning of individual monitoring.

The presence of contamination on surfaces such as floors, bench tops, clothing and skin should be monitored.

2.4.2.4. Air contamination monitoring

Air monitoring should be carried out in areas where airborne contamination may occur owing to disturbance of surface contamination in the vicinity.

2.4.2.5. Personnel monitoring

The radiation exposure due to internally deposited radionuclides (such as inhalation and ingestion) should be determined periodically by either whole body counting or monitoring of excreta such as urine or by scanning a particular organ. In the case of an incident resulting in widespread contamination of the laboratory environment, workers should be asked to provide urine samples for immediate analysis.

2.4.2.6. Ventilation

Proper ventilation (shielded laminar flow hood) should be maintained in the radiochemical laboratories in which unsealed radioactive materials (i.e. gas or liquid) are handled, when the activity level is between 1 and 10 times the maximum recommended body burden.

Glove boxes should be used when there is a potential hazard of contaminating the working environment or of exposing the worker to more than 10 times the recommended maximum body burden or whole body dose limit.

2.4.2.7. Shielding and radioactive waste handling

Shielding should be provided for safe working conditions. The amount of shielding required depends on:

— Radiation type, quantity and energy;
— Dimensions and radioactivity of the sources;
— Dose rate considered acceptable for radiation workers.

Gamma radiation can be effectively shielded by using lead bricks or lead pots.

To avoid any serious source of internal and external radiation hazards from radioactive waste, adequate arrangements should be made for the proper collection and safe disposal of all kinds of radioactive wastes generated at the facility.

REFERENCES TO SECTION 2

[2.1] INTERNATIONAL ATOMIC ENERGY AGENCY, Operational Guidance on Hospital Radiopharmacy: A Safe and Effective Approach, IAEA, Vienna (2008).

[2.2] RODRIGUES, M., et al., Labelling of platelets with indium-111 oxine and technetium-99m hexamethylpropylene amine oxime: Suggested methods, Eur. J. Nucl. Med. **26** (1999) 1614–1616.

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[2.4] ROCA, M., et al., Guidelines for the labelling of leucocytes with ^{111}In-oxine, Eur. J. Nucl. Med. Mol. Imaging **37** (2010) 835–841.

[2.5] DE VRIES, E.F.J., et al., Guidelines for the labelling of leucocytes with ^{99m}Tc-HMPAO, Eur. J. Nucl. Med. Mol. Imaging **37** (2010) 842–848.

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[2.7] PALESTRO, C.J., et al., Society of Nuclear Medicine Procedure Guideline for ^{99m}Tc-Exametazime (HMPAO)-Labeled Leukocyte Scintigraphy for Suspected Infection/Inflammation, Version 3.0, SNM, Reston, VA (2004).

[2.8] INTERNATIONAL COMMISSION ON RADIOLOGICAL PROTECTION, Radiation Dose to Patients from Radiopharmaceuticals (Addendum to ICRP Publication 53), Publication 80, Pergamon Press, Oxford and New York (1999).

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3. OPERATIONS

The labelling of autologous cells is a well rehearsed procedure and should not be extemporaneous. It is therefore important to start with sterile, pyrogen free, GMP grade materials and disposables, and to perform all labelling steps using aseptic procedures while working in a suitable environment. This section analyses all the necessary requirements for handling patient blood, performing isolation and the in vitro radiolabelling of autologous cells.

3.1. OPEN AND CLOSED SYSTEMS

Aseptic procedures should be used during blood cell isolation and labelling. With this in mind, several closed systems have been developed in recent years which aim to optimize sterile conditions. Indeed, the rapidly increasing awareness of the need to adhere to strict quality and safety guidelines is making blood cell purification and labelling procedures difficult to perform in many nuclear medicine centres that are not equipped with the necessary facilities, thus limiting the diffusion of such a useful procedure.

3.2. FACILITIES

3.2.1. Laboratory requirements

Requirements for the laboratories used for cell purification and labelling may vary from country to country, but they generally follow the same regulations as the preparation of radioactive kits for clinical nuclear medicine. The laboratory should have defined specifications for cleanness, laminar flow hood, particulate and microbiological contamination.

3.2.2. Equipment

The necessary equipment includes:

(a) Sterile laminar flow hood: This is a class IIb down flow laminar flow hood equipped with a UV lamp and high efficiency particulate air filter, which needs to be regularly controlled.
(b) Centrifuge: This is used for cellular separation at a well defined g rate that is characteristic for each cell type. It should have a variable angle rotor

and be preferably refrigerated. The centrifuge buckets need to have caps or closures to minimize the risk of blood contamination of the internal parts of the centrifuge in case of accidental blood tube breakages.

(c) Optical microscope: This is required for viability tests and microscopic examination of samples and should have a 10x, 20x and 40x optical magnification.

(d) Isotope calibrator: The well counter dosimeter is necessary to measure radioactivity in cells and supernatant. All nuclear medicine centres are equipped with such a dosimeter.

(e) Vortex: This is quality control requirement rapidly and efficiently shakes a sample or pellet.

(f) Heated water bath: This is required for the radiolabelling at standardized temperatures for quality control (including denaturation of red blood cells and limulus tests).

3.3. MATERIALS

3.3.1. Pharmaceutical grade products

The use of pharmacopoeia defined materials should always be preferred to non-pharmaceutical grade substances.

3.3.2. Non-pharmaceutical grade products

The safe use of substances of unknown pharmaceutical quality are only possible if some minimal requirements are followed. In appropriate cases, further qualification of the products will be ascertained by the local hospital pharmacist through a certificate of analysis, testing of the substances and producing in-house monographs.

The final product or labelled cells need to be examined carefully for:

— Preservation of the sample's identity throughout the procedure;
— Appearance (solid, liquid, lyophilized and colour);
— Chemical concentration and composition;
— Chemical impurities;
— Sterility, apyrogenicity and contamination;
— Excipients;
— pH;
— Adherance to temperature specifications;
— Storage conditions;

— Reconstitution and dilution instructions;
— Quality control of the final product;
— Handling instructions if the injection to the patient is delayed.

3.3.3. Disposables

Disposable materials (syringes, vials, tubes, working trays, pipettes, pipette tips, needles, gloves and sterile kwills) should never be re-used, even if they are re-sterilized, set aside per patient and assigned after the labelling procedure, or not used at all. All disposable materials should be discarded, as they can potentially be contaminated.

Stability of the anticoagulant in the syringe may be affected by the presence of lubricants in the syringe. To avoid this problem, the type of syringe (i.e. plastic or glass) should be taken into account, as cells may adhere to a varying extent or clotting may even occur. The labelling efficiency of red blood cells using the in vitro modified method can also be impaired by lubricants. Some syringes do not have lubricants and thus would be preferable for cell labelling. In addition, overspraying of disposable items used during the blood cell labelling procedure with disinfectant solutions such as isopropyl alcohol should be avoided, since if excess alcohol enters the blood sample, it activates white blood cells and affects radiolabelling. All disposable items are wiped with disinfectant swabs and transferred inside the laminar flow hood or the isolator in use.

The quality control of needles and needle cones should be taken into account as retroflow of radiolabelled blood cells and skin contamination may occur. Injecting the labelled cells through indwelling catheters should be avoided, as the radiolabelled cells might stick to the line even if extensively flushed with saline. Personnel should be aware of the manufacturer expiration dates and should use disposable materials only in accordance with manufacturer recommendations.

3.4. GOOD RADIOPHARMACEUTICAL PRACTICE

This section contains a concise description of good radiopharmaceutical practice procedures. Further information compiled by the EANM Radiopharmacy Committee on guidelines in current procedures can be found in Ref. [3.1].

3.4.1. Standard operating procedures

The central objective of any SOP documentation is to provide an audit trail from the investigation request to equipment performance, the quality control procedures and administering individual patient activities of

radiopharmaceuticals. Records need to be comprehensive and cover details on staff, patients, radiopharmaceuticals, reagent kits, radioisotopes, facilities, equipment, radiation safety and fire safety [3.2]. References [3.1–3.8] provide a complete guidance about how these documents should be prepared. Furthermore, Appendices I–III and Refs [3.3–3.8] demonstrate various examples for preparation and quality control protocols. The following are the minimum requirements to ensure the audit trail:

— Performing cell labelling according to each individual method;
— Performing quality control;
— Working with instrumentation;
— Disinfecting the clean room and class IIb safety cabinet/isolator;
— Safety procedure in case of accidents.

3.4.2. Procedures

All operations should be performed in accordance with written SOPs, which should contain detailed methodology and comply with local practice. They also need to be authorized and periodically reviewed by the responsible individuals [3.2]. For maintenance purposes, the use of a quality handbook to connect all the different SOPs is advisable.

Accordingly, the following procedures should be available and implemented for the reliable operation of labelled cell preparations:

— Writing and evaluation of procedures;
— Cell labelling from start to finish;
— Validation of materials and methods;
— Supplier qualification;
— Safety for centrifuges;
— General radiation safety;
— Radiation safety under special circumstances (i.e. use of centrifuges);
— Instrument calibration;
— Class IIb safety cabinet or isolator working and calibration;
— Logbooks;
— Auditing;
— Updating procedures and master plan.

3.4.3. Quality standards

The specifications and quality control testing procedures for most of the currently used radiopharmaceuticals are given, for example, in the European Pharmacopoeia, British Pharmacopoeia and the United States Pharmacopoeia.

There should be a written procedure detailing all preparation and quality control data that should be considered before the preparation is dispatched. On every product prior to release, the following parameters need to be assessed:

— Positive identification of the patient who is to receive labelled cells;
— Label needs to be checked for correctness and completeness;
— Total radioactivity;
— Appearance and freedom from gross particulate contamination.

Other strict quality control parameters exist [3.1]:

— Labelling kits and resulting radiolabelled preparations;
— Testing of sterility and bacterial endotoxins;
— Preparations from autologous patients' material;
— Kits prepared in-house.

3.4.4. Audits

All procedures established at a radiopharmacy department should be verified by internal inspections. Internal inspection of the premises should be done at least once a year [3.1, 3.2].

REFERENCES TO SECTION 3

[3.1] EANM RADIOPHARMACY COMMITTEE, Guidelines on Current Good Radiopharmacy Practice (cGRPP) in the Preparation of Radiopharmaceuticals, Version 2, EANM, Vienna (2007).

[3.2] INTERNATIONAL ATOMIC ENERGY AGENCY, Operational Guidance on Hospital Radiopharmacy: A Safe and Effective Approach, IAEA, Vienna (2008).

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[3.4] ROCA, M., et al., A consensus protocol for white blood cells labelling with technetium-99m hexamethylpropylene amine oxime, Eur. J. Nucl. Med. **25** (1998) 797–798.

[3.5] ROCA, M., et al., Guidelines for the labelling of leucocytes with ^{111}In-oxine, Eur. J. Nucl. Med. Mol. Imaging **37** (2010) 835–841.

[3.6] DE VRIES, E.F.J., et al., Guidelines for the labelling of leucocytes with ^{99m}Tc-HMPAO, Eur. J. Nucl. Med. Mol. Imaging **37** (2010) 842–848.

[3.7] PALESTRO, C.J., et al., Society of Nuclear Medicine Procedure Guideline for ^{111}In-Leukocyte Scintigraphy for Suspected Infection/Inflammation, Version 3.0, SNM, Reston, VA (2004).

[3.8] PALESTRO, C.J., et al., Society of Nuclear Medicine Procedure Guideline for ^{99m}Tc-Exametazime (HMPAO)-Labeled Leukocyte Scintigraphy for Suspected Infection/Inflammation, Version 3.0, SNM, Reston, VA (2004).

4. REQUIREMENTS FOR RADIOLABELLING AND QUALITY CONTROL OF WHITE BLOOD CELLS

This section provides basic rules for white blood cell labelling and some basic regulations for the quality of radiopharmaceuticals. The European Pharmacopoeia and the United States Pharmacopoeia were used as sources for these standards. This section deals with the basic quality requirements for radioisotopes used for white blood cell labelling purposes. Furthermore, a non-limitative listing is made for the available radiopharmaceuticals used for cell labelling.

4.1. CONSIDERATIONS IN THE USE OF RADIOISOTOPES AND CHELATORS

4.1.1. Technetium generators

For the quality of a pertechnetate solution, it is assumed that molybdenum was produced from a nuclear reactor (i.e. a fission originated molybdenum). However, other molybdenum (Mo/Tc) generators are available from non-fission molybdenum. The quality standards for these are fully covered by the requirements for fission produced Mo/Tc generators. The main standards for the pertechnetate injection are listed in Table 4.1.

4.1.2. Indium compounds

The most common way to obtain ^{111}In is proton irradiation on a cadmium target: ^{111}Cd(p, n)^{111}In or ^{112}Cd(p, 2n)^{111}In. It is manufactured as a trichloride salt in a chlorhydric acid solution. This form is used as a precursor and is described in pharmacopoeia monography. For white blood cell labelling, ^{111}In is complexed to oxine, and the manufactured solution presents radiopharmaceutical qualities (see Fig. 4.1). This solution needs to be buffered with tris(hydroxymethyl) aminomethane (TRIS) before it is used for cell radiolabelling. The user can verify radionuclidic purity by gamma spectrometry (indium gamma rays are 23 keV (69%), 171 keV (90%) and 245 keV (94%)) and radiochemical purity by instant thin layer chromatography in silica gel (ITLC-SG). The most common solvent used for this purpose is a mixture of dichloromethane–methanol (8:2). In this condition, the retention factor (Rf) of ^{111}In-oxine is in the range of 0.5–0.8 and the Rf of ^{111}In colloids is 0. To verify the ability of the ^{111}In-oxine complex to penetrate the cell membrane, it is possible to practise an extraction test with

TABLE 4.1. MAIN QUALITY REQUIREMENTS FOR ^{99M}TC-PERTECHNETATE ELUTION SOLUTION AND THE GENERATOR FROM WHICH IT COMES

Criterion	Eur. Pharm. (6th edn)[a]	USP (30th edn)[b]	General standards	In-house testing recommended?
Radioactivity	90–110% of the declared amount should be present	90–110% of the declared amount should be present		Yes, for each elution
Mo breakthrough (main impurity risk)	Mo-99 < 0.1%	Mo-99 < 0.015%		Yes, after the first elution on each day
Sterility and pyrogen test	Microorganism and endotoxin free	Microorganism and endotoxin free		To be tested on final preparation (optional)
Isotonicity	No requirement	Isotonic		No, this control is under the responsibility of the manufacturer
Al test	<5 μg/mL	<10 μg/mL		Yes, after the first elution on each day
Radiolysis impurities	None	None	TcO_4^- > 95%[c]	Yes, for each elution
Other impurities (%)	I-131 < 5×10^{-3}	Total of γ emitters (Mo excluded) < 0.05		No, this control is under the responsibility of manufacturer
	Ru-103 < 5×10^{-3}	Ru-103 < 5×10^{-3}		
	Sr-89 < 6×10^{-5}	Sr-89 < 6×10^{-5}		
	Sr-90 < 6×10^{-6}	Sr-90 < 6×10^{-6}		
	Other γ emitters < 0.01	Other γ emitters < 0.01		
	α emitters < 1×10^{-7}	α emitters < 1×10^{-7}		

TABLE 4.1. MAIN QUALITY REQUIREMENTS FOR ^{99M}TC-PERTECHNETATE ELUTION SOLUTION AND THE GENERATOR FROM WHICH IT COMES (cont.)

Criterion	Eur. Pharm. (6th edn)[a]	USP (30th edn)[b]	General standards	In-house testing recommended?
Appearance	Clear, colourless solution	Clear, colourless solution		Yes, for each elution
Half-life	6.02 h, emitting γ radiation	6.02 h, emitting γ radiation		Optional test
γ ray spectrum	Major peak at an energy of 0.140 MeV	Major peak at an energy of 0.140 MeV		Optional test

[a] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES AND HEALTHCARE, European Pharmacopoeia, 6th edn, Council of Europe, Strasbourg (2007).

[b] US PHARMACOPEIAL CONVENTION, USP NF 2007, 30th edn, USP (2007).

[c] UK RADIOPHARMACY GROUP, UKRG Radiopharmacy Handbook, BNMS, Nottingham (1997).

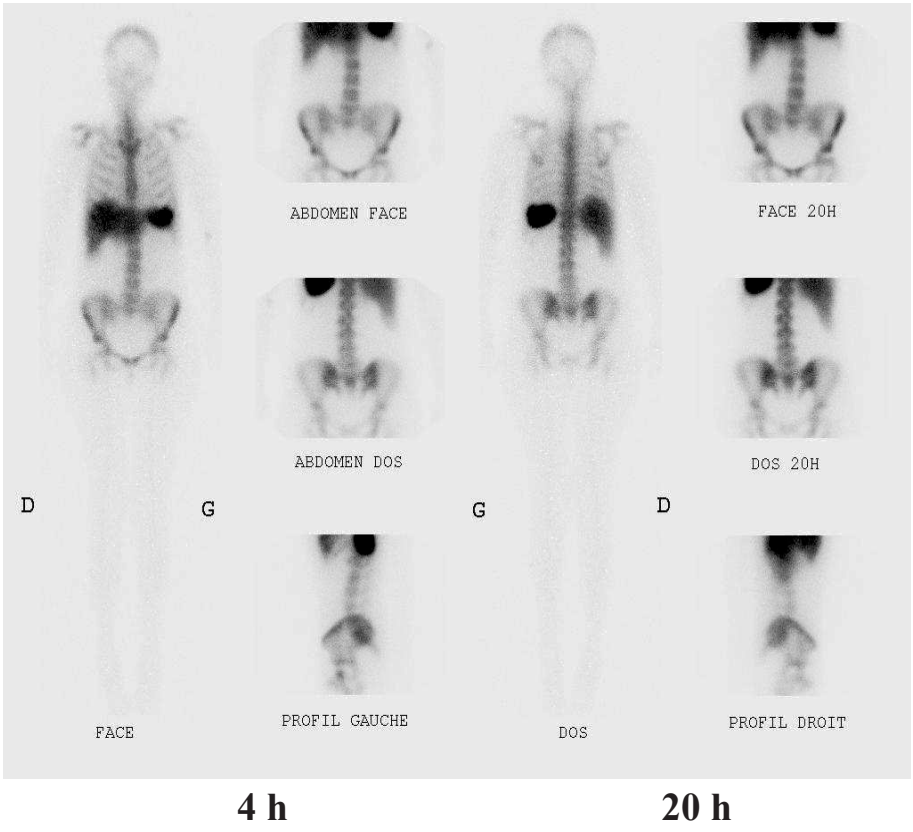

FIG. 4.1. Normal distribution of ^{111}In labelled leukocytes.

acid, water and octanol. The results of radiochemical controls need to be higher than 90%.

4.1.3. Technetium chelators

4.1.3.1. Considerations in the use of HMPAO

Hexamethylpropyleneamine oxime bound to ^{99m}Tc (^{99m}Tc-HMPAO) is the most widely used complex for white blood cell labelling. It is easily obtained by adding a pertechnetate solution (500–1000 MBq) into a vial containing HMPAO and stannous chloride ($SnCl_2$) (500 μg HMPAO and 7.6 μg $SnCl_2$) (see Fig. 4.2). In this condition, reduced technetium forms a neutral pyramidal complex with the technetium oxide core. However, the neutral lipophilic complex (complex I)

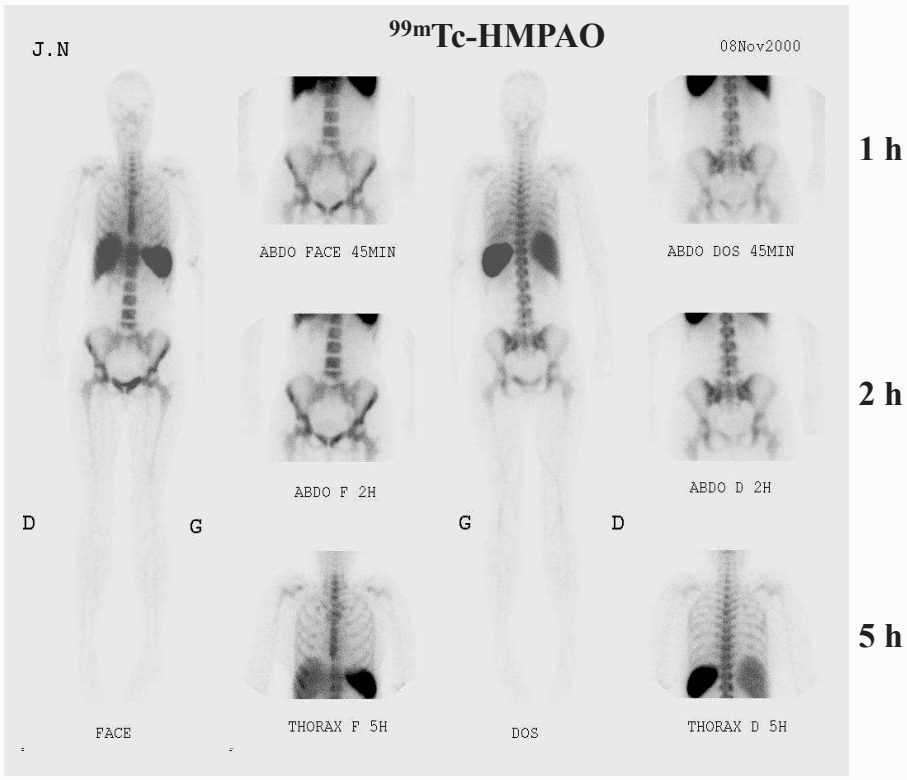

FIG. 4.2. Normal distribution of ^{99m}Tc-HMPAO labelled leukocytes.

changes to a hydrophilic ionic form (complex II) in less than 1 h. This change is faster in alkaline conditions. This modification is used for cell labelling.

Only complex I can pass the cell membrane and in the intracellular higher pH, this complex changes into complex II, which is sequestered locally. The ^{99m}Tc-HMPAO solution needs to be controlled to determine the respective percentage of complexes I and II before being used for cell labelling. The manufacturer recommends a radiochemical control by ITLC-SG with two solvents: butanone and sodium chloride (NaCl). With the first system, complex I and free pertechnetate have an Rf in the range of 0.8–1 and complex II and hydrolysed technetium have an Rf of 0. In the second system, only pertechnetate has an Rf of 1; the other compounds have an Rf of 0. Some other different systems on cellulose paper with ethyl acetate solvent may be used in order to replace the ITLC-SG plates. In this condition, only complex I has an Rf of 1; the other compounds have an Rf of 0.

4.1.3.2. Other complexes

Some other complexes are also used as well for technetium cell labelling or for infection imaging in research. The complexes most frequently found in the literature are hydrazinonicotinamide (HYNIC) and mercaptoacetyltriglycin (MAG-3).

The ^{99m}Tc-HYNIC complex is generally stabilized by the addition of N-tris(hydroxymethyl)methylglycine (tricine). This complex is described for labelling antibodies and for labelling interleukine-8 in Ref. [4.1]. A derivative hydrazido stabilized complex with a nitrido-technetium core is described for lymphocyte and neutrophilic white blood cell radiolabelling without any vector in Ref. [4.2].

Technetium-99m-MAG-3 complexes are generally used for radiolabelling small molecules. For infection imaging, this type of ligand is used with interleukine-2 (IL-2). In this case, the technetium complex is first obtained by reduction of pertechnetate in the presence of the ligand at high temperature (80°C) and low pH (pH2). Afterwards, this complex is fixed to IL-2 at pH9 [4.3].

4.1.4. Indium chelators

4.1.4.1. Considerations in the use of oxine

Oxine forms complexes with many metals (iron, lanthanides and indium). With indium, oxine forms a neutral complex which is transported across cell membranes and retained in the intracellular compartment by fixing on proteins. Compared with technetium labelling, indium cell labelling is more stable.

4.1.4.2. Other ligands

Many other ligands are described for labelling indium to small molecules or antibodies. The most cited ligands are the derivatives of DTPA or cyclododecane-tetraacetate (DOTA). These types of ligand form very stable complexes with indium, but these macrocycle ligands have low kinetics of dissociation favourable to inhibit the in vivo transchelation. For direct cell labelling, only troponate ligands have been used in clinical trials as an alternative to oxinate.

4.2. CONSIDERATIONS IN THE USE OF TECHNETIUM COLLOIDS FOR RADIOLABELLING NEUTROPHILS

In the United States of America, white blood cells are mainly labelled to ^{111}In-oxine; in Europe, white blood cells are mostly labelled with ^{99m}Tc-HMPAO. All methods require the withdrawal of blood from patients, and the first two methods also require the purification of white blood cells from red blood cells, in vitro labelling under sterile conditions and reinjection of labelled cells into the patient. Phagocytosis of radioactive colloids has attracted considerable interest as a simple method for labelling monocytes and granulocytes with gamma emitting radionuclides for clinical studies. Attempts have been made to label leukocytes not only with a ^{99m}Tc-sulphur colloid [4.4, 4.5], but also with colloidal gold [4.6] and labelled millimicrospheres [4.7]. All showed unsatisfactory labelling efficiency or relatively poor stability [4.4]. Schroth et al. first used ^{99m}Tc-SnF_2 colloids for labelling leukocytes in the whole blood [4.8]. Following the administering of labelled cells, sites of occult sepsis have been successfully identified in vivo by gamma camera imaging.

Labelling efficiency is significantly higher when leukocytes are labelled in vitro with ^{99m}Tc-SnF_2 compared with ^{99m}Tc-HMPAO. This can be explained by the different uptake mechanisms. Technetium-99m-SnF_2 is taken up by neutrophils and monocytes by phagocytosis, while ^{99m}Tc-HMPAO is a lipophilic agent that penetrates cell membranes, particularly eosinophils. The size and nature of radiolabelled colloids are important parameters to obtain optimal phagocytosis with minimal surface adsorption [4.7].

Hanna and Lomas describe a labelling efficiency of 98% in whole blood using colloids with an average particle size of 2.1 μm, and demonstrate that the use of smaller particles, present in lyophilized kits, is associated with an increase of free radioactivity in plasma and decreased labelling efficiency [4.9]. To correctly calculate the labelling efficiency, it is necessary to separate free colloids from labelled cells. This is necessary because free colloids migrate with cells and little radioactivity remains in the supernatant using simple centrifugation with a saline solution.

It has been reported that phagocytosis is decreased in children with type 1 diabetes. Furthermore, poorly controlled diabetic patients with type 1 diabetes for less than five years have a lower phagocytic capacity than patients with a longer disease duration but better controlled disease [4.10]. This is related to the high blood glucose levels. An inverse correlation has been found between blood glucose and labelling efficiency for both SnF_2 and HMPAO [4.11]. Another biochemical parameter that seems to influence the viability of cells is the total cholesterol level or LDL cholesterol [4.11]. It has been previously described that high cholesterol levels may induce cell death in a human monocytic blood

cell line by apoptosis [4.12]. This data suggests that blood cholesterol level should also be within the normal range for optimal labelling and viability of labelled cells.

Soon after labelling, ^{99m}Tc-SnF_2 and ^{99m}Tc-HMPAO are both eluted from labelled cells. This phenomenon increases with time, although the amount of radioactivity spontaneously released from cells is significantly higher for ^{99m}Tc-SnF_2 labelled white blood cells than for ^{99m}Tc-HMPAO labelled white blood cells. This may have an important clinical implication. The presence of some free ^{99m}Tc-pertechnetate can be observed in the thyroid and gastric mucosa. Technetium-99m-HMPAO is physiologically excreted into the gastrointestinal tract, and the time at which it is observed as well as its location and extension vary. The maximum sensitivity of abdominal images is therefore 2–3 h after the injection of ^{99m}Tc-HMPAO labelled white blood cells [4.13]. By contrast, radiolabelled colloids can be taken up by reticuloendothelial cells of spleen and liver and to a lesser extent by the bone marrow [4.11]. Abdominal infections have been studied with ^{99m}Tc-SnF_2 and ^{111}In labelled white blood cells, and results show that there was high degree of concordance between the two techniques. There have been two cases with bowel uptake of ^{99m}Tc-SnF_2 labelled white blood cells but not of ^{111}In labelled white blood cells and follow-up excluded abdominal disease [4.14]. This finding can be correlated to colloid uptake by the reticuloendothelial system of mucous membrane. Technetium-99m-SnF_2 labelled white blood cells have also been used to visualize musculoskeletal and soft tissue melioidosis [4.15]. Thus, ^{99m}Tc-SnF_2 labelled white blood cells could be helpful in the study of infections in organs or tissues where ^{99m}Tc-HMPAO labelled white blood cells have low sensitivity.

In summary, in vitro labelling efficiency of purified autologous leukocytes is significantly higher with ^{99m}Tc-SnF_2 than with ^{99m}Tc-HMPAO. Both methods are non-toxic for labelled cells over a period of 4 h. The percentage of spontaneously released ^{99m}Tc-SnF_2 is significantly higher compared with ^{99m}Tc-HMPAO, but ^{99m}Tc-SnF_2 is not excreted into the gastrointestinal tract. This suggests a clinical role in investigating abdominal inflammation and infections, although in vivo comparative studies are necessary to evaluate the accuracy of ^{99m}Tc-SnF_2 labelled white blood cell scintigraphy compared with other well standardized techniques. The use of ^{99m}Tc-SnF_2 for leukocyte labelling could also be attempted in leukocyte rich plasma, thus avoiding leukocyte purification.

4.3. CONSIDERATIONS IN THE USE OF ANTICOAGULANTS

There are several anticoagulation agents available, but the two most commonly used are acid citrate dextrose type A anticoagulant (ACD-A) and

heparin. ACD-A solution is composed of citric acid, sodium citrate, dextrose and water, and it inhibits coagulation by binding free calcium in the blood. ACD-A is preferred because it prevents the adherence of proteins and blood cells to plastic containers, resulting in increased leukocyte recovery. Citrate has also been noted to inhibit leukocyte degranulation and stress oxidation. In addition, it has been reported that the degree of leukocyte deformability — a property necessary for leukocytes to leave the pulmonary circulation — is reduced in the presence of calcium, thereby making ACD-A solution a better agent, especially when blood is to be transported outside the nuclear medicine department for processing. The amount of ACD-A required is usually 1 mL ACD-A for every 6 mL of blood.

The second anticoagulant is the polysaccharide heparin, which in itself has no anticoagulant activity. However, when it combines with a naturally occurring anticoagulant cofactor in the blood, antithrombin III, the result is an instantaneous amplification of anticoagulation through the binding of activated clotting factors and the prevention of thrombin formation. Anticoagulation can be achieved with 1 mL (1000 IU) preservative free heparin for 30–50 mL whole blood.

Since the preparation of autologous leukocytes for radionuclide labelling includes several steps of cell washing prior to reinjection of the radiolabelled product, both of these agents are useful and safe for ex vivo anticoagulation. In the United States of America, heparin is used more frequently because it is less expensive and more readily available commercially.

4.4. CONSIDERATIONS IN THE USE OF SEDIMENTATION AGENTS

Most radiopharmaceuticals used for cell labelling are non-selective. Therefore, leukocytes need to be separated from erythrocytes and platelets. Erythrocyte sedimentation — through spontaneous gravitational settling — is the most popular method of removing a large number of red blood cells from the plasma. This simple process can be accelerated by adding aggregation or sedimentation agents. These agents are generally large molecules that promote rouleaux formation of red blood cells. Sedimentation agents include 6% dextran in saline (1 part to 5 parts blood), 2% methylcellulose in saline (1 part to 10 parts blood), 6% hydroxyethyl starch (HES-6) (1 part to 5 parts blood) or 10% hydroxyethyl starch (HES-10) (1 part to 5 parts blood). The final concentration should be indicated (i.e. concentration as a percentage after mixing with blood).

Dextran is a branched polysaccharide of glucose formed by the bacterium *Leuconostoc mesenteroides*, which renders this agent antigenic. This product has been known to prevent platelet aggregation. Improved preparation has reduced hypersensitivity reactions.

Methycellulose is a non-toxic and non-allergenic glucose polymer. Used as 2% methylcellulose, it has been reported to give rise to a yield of around 70% neutrophils, 26% lymphocytes, 9% monocytes and 24% red blood cells in the supernatant. Neutrophil viability is high at 75%. Nearly 100% yield of neutrophils has also been reported with 1% methylcellulose coupled with low speed centrifugation at 20g for 10–14 min.

Hydroxyethyl starch (HES), a synthetic molecule similar to glycogen, is eliminated from the body after injection. It has been associated with more rapid red blood cell sedimentation, greater leukocyte recovery, and the absence of toxic or allergic reactions. An additional advantage is its ability to prevent the aggregation of granulocytes. HES-6 and HES-10 are both relatively inexpensive compared with other agents utilized for red blood cell sedimentation. HES-10 is recommended.

Ensuring purity and sterility of any sedimentation agent to be used is highly important in clinical practice. HES usually comes in a 500 mL bags. If this is the case, safe practice includes strict sterile withdrawal of fluid from the bag and the use of disposable syringes for each specimen handled. When 500 mL bottles are available from the hospital pharmacy, HES should be aliquoted in small sterile vials to avoid repeated puncture of the bottle seal. In addition, sterility and pyrogen tests should be periodically performed on the aliquoted material. No blood should remain at the tip of the syringe to avoid coagulation activation and contamination with red blood cells when transferring the cell rich plasma from the syringe. When the sedimentation is very slow, diluting with 5–10 mL of plasma expander and dividing the blood in two to four 50 mL Falcon vials (each with 15 mL of blood) increase the overall sedimentation surface.

4.5. CONSIDERATIONS IN THE USE OF WASHING MEDIA

There are several fluids that can be used to wash labelled leukocytes. It is important to make certain that the fluid will not lead to lysis of leukocytes and will not cause undue activation of neutrophils.

The simplest washing agent that can safely be used is physiologic 0.9% NaCl (saline) or isotonic phosphate buffered saline (PBS) solution (pH7.4). Both are cheap, isotonic, preserve the integrity of the cell membrane and are widely available. Reconstitution of labelled leukocytes with physiologic normal saline or PBS for reinjection poses no changes in a patient's hemodynamic state and has been shown to have no deleterious effects on the functionality of labelled leukocytes.

Hank's balanced salt solution (HBSS) is another safe fluid to wash the cells. It is slightly more costly than physiologic normal saline. If GMP solutions

for human use are not commercially available, they can be formulated in the pharmacy under sterile GMP conditions.

4.6. CONSIDERATIONS IN THE USE OF STABILIZERS

Indium-111-oxine and ^{99m}Tc-HMPAO labelled leukocytes have been used to image inflammation and infection for many years. The advantages of ^{99m}Tc labelled leukocytes include optimal photon energy for gamma camera detection, high resolution images and the ability to detect abnormalities within a few hours after injection. The disadvantages include a lower labelling efficiency than what is obtained with ^{111}In-oxine and an instability of the labelling. Both labelling efficiency and labelling stability can be improved by using stabilized ^{99m}Tc-HMPAO, prepared according to the manufacturer's instructions, using methylene blue combined with a PBS and fresh eluate. A labelling efficiency of approximately 88% has been reported with stabilized ^{99m}Tc-HMPAO, with little or no bowel or urinary tract activity up to 2 h after injection. No significant increase in specific organ and whole body dosimetry estimates has been noted compared with previous estimates using non-stabilized ^{99m}Tc-HMPAO labelled leukocytes [4.16]. The deep blue colour of stabilized ^{99m}Tc-HMPAO makes it difficult to separate the supernatant from the leukocyte pellet, and the so called 'double dilution' technique should be used [4.17]. It is important to note, however, that data on the use of stabilized ^{99m}Tc labelled white blood cells, including effects on the cells, are limited.

4.7. CONSIDERATIONS IN THE USE OF DISPOSABLES

Sterility of labelled blood products is of paramount importance. Glassware is economical because it can be washed, autoclaved and reused. However, these containers are also breakable, posing the danger of spillage and loss of blood products. The additional costs of washing agents and autoclaving may further discourage the use of glassware. Plastic disposables have come a long way. Although some plastic disposables can be autoclaved and reused, they are generally manufactured as sterile, single use kits. They are more resistant to breakage and thus preferred for the preparation of labelled blood products. Both plastic and glass are manufactured to limit cell and protein adhesion to their inner surface.

4.8. CONSIDERATIONS IN THE USE OF CENTRIFUGES

Several methods of centrifugation including density gradient and discontinuous density gradient have been used in an effort to separate more efficiently leukocytes from the blood. Simple centrifugation of anticoagulated blood treated with a sedimentation agent is adequate for harvesting leukocytes for labelling in most circumstances.

Simple centrifugation at ambient temperature allows further isolation of leukocytes from other cells in the blood. The supernatant obtained after gravitational sedimentation contains a leukocyte rich fluid admixed with platelets and contaminated red blood cells. This fluid is centrifuged at 450g for 5–10 min, with the lighter platelets afloat in the plasma fluid while leukocytes and red blood cells are pelleted at the bottom of the centrifuge test tube. This pellet is resuspended in physiologic normal saline. The process of centrifugation resuspension is repeated before the resulting leukocyte mixture is incubated with the radionuclide. Prior to final preparation, the labelled leukocytes undergo a series of centrifugation resuspension to remove unbound radionuclide. Some investigators have used a centrifuge speed of 150–200g for 10 min with comparable results.

To ensure that centrifugation conditions are suitable for the successful separation and preservation of leukocytes, it is necessary to know the relative centrifugal force (RCF) expressed in units of acceleration due to gravity (g). Many centrifuge machines only show settings for rotor speed at revolutions per minute (RPM). Consequently, the relationship between RPM and RCF needs to be known by anyone involved in leukocyte labelling. The formula to convert g to centrifuge rotor speed (RPM) is:

$$g = (1.118 \times 10^{-5})\, R\, S^2 \qquad (4.1)$$

where R is the radius of the rotor (in cm) and S is the speed of the centrifuge (in RPM).

4.9. QUALITY CONTROLS

4.9.1. In vitro quality controls

In vitro quality control is an essential part of the procedure of labelling white blood cells. Quality controls may vary according to national rules or recommendations. There are usually routine tests that should be performed at every labelling and periodical controls carried out on particular occasions.

Routine tests need to be easy and quick to perform in order to provide an immediate answer on the injectability of the final product [4.18, 4.19].

These include:

— Labelling efficiency;
— Optical examination of labelled cell suspension.

Periodical tests should be performed for process validation and periodically (every six months) for revalidation of the labelling process:

— Observation under optical microscope;
— Trypan blue exclusion test;
— Cell recovery test;
— Test of radiopharmaceutical elution from cells;
— Sterility tests;
— Pyrogen tests.

The tests are described in Appendix III, with normal values and limits for acceptability.

4.9.2. In vivo quality controls

Several quality controls can be performed in vivo after injecting radiolabelled white blood cells. These include:

— Presence of lung activity after 30 min.
— Evaluation of liver to spleen radioactivity ratio.

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5. REQUIREMENTS FOR RADIOLABELLING AND QUALITY CONTROL OF RED BLOOD CELLS

Red blood cells are found in large numbers in the circulatory system, are easily harvested and are relatively resistant to physical and chemical stimuli that would damage other cells. Therefore, in contrast to radiolabelling white blood cells and platelets, radiolabelling red blood cells is a relatively easy procedure. For imaging purposes, ^{99m}Tc is the most frequently used radionuclide. Due to the fact that ^{51}Cr does not emit suitable gamma photons for imaging, it is only used for non-imaging procedures. Indium-111 can be used if a study is expected to take a few days to complete. Although labelling red blood cells is an easy procedure, optimal labelling of the blood pool is essential for diagnostically adequate studies. Several clinical conditions and drugs have been described that interfere with the radiolabelling of red blood cells [5.1–5.4], and a thorough clinical history is important to exclude these possible sources of poor labelling. If there is a strong clinical suspicion that the latter may be the case, then the in vitro method is preferable. This not only allows calculation of the labelling efficiency, but also the possibility to wash the cells before administering them to the patient.

Red blood cell labelling techniques with ^{99m}Tc evolved from ex vivo labelling methods to in vivo labelling methods aided by the availability of commercial kits. In vivo labelling methods do not require blood sampling, but the images obtained are not always as optimal as those obtained with in vitro labelling techniques. Attempting to optimize this technique with a modified in vivo labelling procedure has also been proposed. This technique is frequently called 'in vivitro' compared to the in vivo and in vitro labelling techniques. After the pretinning in vivo, a red blood cell sample is allowed to react with ^{99m}Tc in a syringe for a sufficient time to provide a stable labelling yield and improved image quality.

Using the in vivo labelling technique, and after intravenously administering ^{99m}Tc-pertechnetate in patients pretreated with stannous ions (Sn^{2+}), the stable labelling of red blood cells is achieved 30 min post-injection. This stable bonding is imperative, as acquisition times up to 24 h are required in studies to detect gastrointestinal bleeding. The ^{99m}Tc labelled red blood cells are cleared slowly from the blood pool, which allows late imaging of occult bleeding sites. In contrast, denatured red blood cells are cleared from the blood pool within 2 h after intravenous injection. Pharmacokinetic modelling shows rapid blood clearance ($T_{½}$ = 6 ± 5 min) for damaged cells. The splenic plateau is reached at 30 min, thus it is appropriate to begin imaging at that time. More than 80% of the injected activity is found in the spleen 2 h after injection [5.5]. As for the other labelled blood cells, it is equally important to adhere to strict quality control procedures

when labelling red blood cells and to ensure correct patient identification when in vitro labelling techniques are used. If good labelling efficiency is essential, the in vitro labelling technique, or the in vitro technique, should be considered.

5.1. CONSIDERATIONS IN THE USE OF RADIONUCLIDES AND CHELATORS

All reagents should be used according to the manufacturer's instructions. The pH of the labelling solution should be verified and the stannous/total tin assay should be conducted to ensure high labelling efficiency.

5.1.1. Technetium-99m

Erythrocytes can be labelled with ^{99m}Tc using several methods. Technetium in the 7^+ oxidation state (pertechnetate) permeates the cell membrane in both directions. To remain bound inside the red blood cell, ^{99m}Tc needs to be reduced. This is achieved with stannous ions such as stannous pyrophosphate (PYP) [5.6]. The original $SnCl_2$ method required washing steps to provide a suitable intravenous solution to be injected. This disadvantage was overcome by adding sodium hypochlorite (NaOCl) and ACD-A. The most widely used is the kit of Smith and Richards [5.7], originally known as the Brookhaven National Laboratory (BNL) kit, subsequently marketed by Covidien as the UltraTag RBC kit [5.8]. Technetium-99m attaches to the globin moiety of the haemoglobin in the red blood cell [5.9].

5.1.1.1. Technetium generators

Some chromatographic generator sources of pertechnetate may contain chemical additives which could interfere with certain labelling systems. If sodium pertechnetate is obtained from a source other than the one suggested in the kit's package insert, the compatibility of the red blood cell labelling kit with that pertechnetate should be validated.

The chemical effect of the mass of technetium should also be considered in the preparation of the radiopharmaceutical agents. The source of pertechnetate should be an eluate from a generator that has been eluted no later than 24 h earlier. This will prevent the presence of excess ^{99}Tc, which is not useful for imaging but will compete for stannous ions, resulting in low labelling efficiency [5.10]. As the amount of tin in the kit for in vitro labelling is very low, it is recommended that the first eluate of the generator is not used for the labelling, as it may lead to poor labelling efficiency.

Sterility of the generator is imperative. It should be stored in an area with maximum surrounding control of environmental sterility (quality assurance of air). The eluates should be sterile for daily use, so general measures should be applied to keep the generator needles sterile during the generator's use. The pertechnetate vials need to be labelled with the date, time and activity. Adequate quality control tests for radiochemical purity should be performed.

5.1.1.2. Technetium chelators: Considerations in the use of PYP, NaOCl and ACD-A

Initial methods of labelling red blood cells with ^{99m}Tc were in vitro techniques involving repeated centrifugation and washing steps [5.11]. One of the first successful methods using $SnCl_2$ was described by Eckelman et al. [5.12]. Altered biodistribution of ^{99m}Tc because of intravascular activity was first reported in 1975 in a brain scan by Chandler and Schuck [5.13]. The patient had previously undergone a bone scan, and the technetium activity was increased in erythrocytes, suggesting in vivo reduction of pertechnetate and labelling of intracellular molecules in the erythrocytes. This interaction was the starting point of the in vivo labelling of red blood cells using PYP as the source of stannous ions.

Tin (Sn(II) or Sn^{2+}) is necessary for the 'pretinning' of red blood cells, in order to reduce the ^{99m}Tc once pertechnetate has entered them. Free pertechnetate freely diffuses in and out of the cells, while the reduced ^{99m}Tc is bound to haemoglobin and thus retained in the cells [5.9]. The most frequently used forms of tin are stannous citrate and PYP. The best labelling yield of the red blood cells is with a Sn(II) concentration of at least 10 μg/kg [5.14]. A lower amount of Sn(II) will result in suboptimal binding efficiencies.

Products containing 1 mg of stannous chloride dihydrate per vial are the most convenient for red blood cell labelling using in vivo and modified in vivo procedures. If multiple procedures are attempted with vials containing more $SnCl_2$, the possibility of oxidation of the stannous ions, which will result in poor labelling, needs to be kept in mind. Patient medication may interact with the stannous ions (see Section 5.6 for details). PYP should not be given through heparinized catheters or intravenous dextrose lines because fewer stannous ions will be available with a resultant diminished labelling efficiency of red blood cells [5.15].

In the in vivo labelling method, the interval between the first intravenous injection of cold PYP and the pertechnetate should be 30 min to obtain >95% of activity associated with the red blood cells.

Surplus extracellular stannous ions may result in the reduction of ^{99m}Tc to a form which is unable to penetrate the cells [5.16]. This may lead to poor

labelling efficiency. As it is not possible to get rid of the excess stannous ions during in vivo labelling, it is especially important to keep the amount of tin low.

When red blood cells are labelled in vitro, a NaOCl solution can be used to oxidize the extracellular tin before the pertechnetate incubation. This causes the extracellular stannous ions to oxidize to the stannic form (Sn^{4+}), while the intracellular stannous ions will not be affected. Labelling efficiencies of >95% have been reported if less than 1 mL of a 0.1% NaOCl solution is added. Higher volumes lead to haemolysis of the cells [5.17]. This technique is used in the commercial in vitro kit (UltraTag RBC). The chemical oxidation can be performed in the whole blood, therefore the centrifugation step, which can damage the cells while labelling, is spared.

ACD-A can also be added to sequester any residual extracellular stannous ions, as the citrate removes the plasma bound tin as tin citrate, making them more readily available for oxidation by NaOCl [5.18].

5.1.2. Indium-111

The ^{111}In labelling of red blood cells is described in the literature [5.19], but it is rarely used clinically. It is manufactured as a trichloride salt in a hydrochloric acid solution. This form is used as a precursor for radiolabelling and is described in several pharmacopoeias. For labelling red blood cells, indium is complexed using tropolone or oxine. Tropolone is not available as a licensed kit. Depending on the manufacturer, the $^{111}InCl_3$ solution may be supplied as a radiopharmaceutical or a radiochemical. In the latter case, the solution may not meet all pharmaceutical quality criteria and the labelling procedure needs validation and thorough quality control before use. Indium-111-oxine is used in a solution buffered with either TRIS or N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer. Indium-111-oxine solution is commercially available as a radiopharmaceutical product containing polysorbate 80 and HEPES buffer.

No differences in the in vitro labelling behaviour, viability or clinical imaging have so far been discovered for oxine or tropolone, irrespective of the radiotracer used. As oxine is by far more widely available, most studies have been performed using this complex. Indium-111-oxine once released from cells is readily re-utilized and bound to plasma proteins, mainly transferrin. Due to this high protein binding affinity, the incubation medium for radiolabelling needs to be plasma protein free.

For intermittent or small intestinal bleeding, ^{111}In-oxine labelling may be considered and be advantageous, allowing scintigraphic monitoring whenever needed for up to one week and increasing likelihood of detection. Stool counting can be done in parallel.

5.1.3. Chromium-51

For the labelling of red blood cells, ^{51}Cr-sodium chromate is regularly used. A 16 mL sample of whole blood is taken from the patient, premixed with 4 mL ACD-A solution and then mixed with a patient activity of ^{51}Cr-sodium chromate solution. The sample is then incubated at 37°C for 10 min and is ready to be administered to the patient after performing the quality control described in Section 5.8.

5.2. CONSIDERATIONS IN THE DENATURATION OF RED BLOOD CELLS

Circulating senescent erythrocytes have an important role in the estimation of splenic reticuloendothelial function, as damaged red blood cells and cellular debris are sequestered by the spleen when phagocytized by the macrophages (sinusoids and reticular cells). Chromium-51-sodium chromate labelled erythrocytes became the basis for the determination of the distribution of tagged red blood cells, first utilized by Gray and Sterling [5.20], and the determination of the sites of sequestration. Labelled cells concentrate ^{51}Cr in the cytosol and the labelling is relatively stable, with only 1% elution per day. This makes ^{51}Cr more efficient and reliable than ^{111}In for erythrocyte labelling [5.19]. Since ^{51}Cr is not suitable for gamma camera imaging and ^{111}In is not suitable because of its long half-life, ^{99m}Tc labelled red blood cells came into practice. The red blood cells are first labelled with ^{99m}Tc according to established methods, and then denatured, or first damaged, and then radiolabelled [5.21].

Denatured erythrocytes accumulate in the spleen and are used as an index of splenic function. Alteration of the red blood cell membrane is the required step in preparing the cell for spleen specific imaging. This can be achieved by immunological sensitization or chemical denaturation, yet damaging the cellular membrane through heat is most commonly used. When red blood cells are denatured, spherocytes form which are characterized by a loss of intracellular electrolytes and a more fragile membrane that becomes very susceptible to cell lysis and sequestration, contributing to the spleen scintiscan [5.22]. Technetium-99m labelled heat damaged red blood cell scanning is a simple and useful technique for splenic imaging.

The recommended temperature is 49.5°C, with gentle agitation in a water bath, and incubation for a maximum of 15–20 min [5.5, 5.11, 5.14, 5.19, 5.23]. If the patient's haematocrit is low, it is possible to increase the incubation time by 2–5 min. After labelling, the blood needs to be gently inverted several times

in the syringe. Visible clots should be avoided, as they will produce hot spot artefacts in the image.

If the heating temperature is too low or the heating time is inadequate, there will be less uptake by the spleen and more blood pool activity. If the heating temperature is too high or the heating time is too long, spleen uptake will also diminish but liver uptake will be higher [5.24]. A small amount of blood (1–3 mL) should be used to enable more uniform heating of the cells and not to saturate the splenic cell sequestration capacity of the spleen, leading to high blood pool activity [5.5].

5.3. CONSIDERATIONS IN THE USE OF ANTICOAGULANTS

If an intravenous line is used in the patient for the in vitro and in vivo modified technique, it should be anticoagulated with a heparin solution of 10 units/mL (e.g. 100 units in 10 mL saline).

There are different opinions on the effect of the different anticoagulants on the labelling efficiency of the red blood cells. Porter et al. describe both heparin and ACD-A as anticoagulants in the labelling of red blood cells, with a reported labelling efficiency of 93% using ACD-A compared with 87% for heparin [5.10]. Bernardo-Filho et al. are not able to demonstrate any difference in the labelling efficiency between heparin and ACD-A [5.25]. Excess ACD-A impairs the diffusion of stannous ions across the red blood cell membrane [5.26], with a reduction in labelling efficiency. With EDTA, the percentage of radioactivity incorporated into the red blood cells is smaller than the other anticoagulants because EDTA sequesters ^{99m}Tc before it can enter the red blood cells [5.25].

5.4. CONSIDERATIONS IN THE USE OF WASHING MEDIA

After labelling the red blood cells, they are resuspended in 0.9% NaCl solution, which is readily available and compatible with them and can also be used for the washing of these cells. It is not necessary to use buffered solutions for the washing of red blood cells.

5.5. CONSIDERATIONS IN THE USE OF DISPOSABLES

Disposable materials (syringes, vials, tubes, working trays, pipettes, pipette tips, needles, gloves and sterile kwills) should never be re-used. All disposables for each labelling are set aside per patient and discarded immediately following

the labelling procedure to avoid contamination. Further information on the proper use and handling of disposables can be found in Section 3.3.3.

5.6. DRUG INTERFERENCE AND PATIENT FACTORS INFLUENCING CELL LABELLING

Several drugs are known to interfere with the radiolabelling of red blood cells, and these effects have been summarized in a number of publications [5.1, 5.2, 5.27–5.29]. Some of the interactions include:

— Heparin competes with red blood cells for stannous ions.
— Chemotherapy disrupts the red blood cell membrane.
— Methyldopa and hydralazine both produce the oxidation of Sn^{2+} to Sn^{4+}.
— Digoxin and calcium channel blockers interfere with cellular uptake of stannous ions.
— Quinidine and dipyridamole block transmembrane transport.

Cyclosporine is reported to interfere with cell labelling [5.30]. Later investigations, however, show that this effect only occurs when very low concentration of stannous ions are used [5.31–5.33].

Many patient related factors can also influence red blood cell labelling. Summaries can be found in Adalet and Cantez [5.1] and Hambye et al. [5.27].

If the haematocrit value is low, ^{99m}Tc is reduced outside the red blood cells, leading to low labelling efficiency. Immune disorders, a combination of multiple pathologies and red blood cell antibody formation are also reported to influence labelling efficiency.

5.7. CONSIDERATIONS IN THE USE OF CENTRIFUGES

In the in vitro labelling method, the extracellular stannous ions are removed by centrifugation, a physical step that separates the stannous treated red blood cells from the non-cell-bound stannous ions. For labelling, red blood cell centrifuges do not require refrigeration and generally do not require high speed capabilities. It is imperative that centrifuges are located in a clean area in which the cell labelling process is taking place [5.34].

In vivo and modified in vivo techniques do not need centrifugation. If the UltraTag RBC kit is used to label red blood cells, NaOCl oxidizes the extracellular stannous ions and a centrifuge is not required.

5.8. QUALITY CONTROL

The labelling efficiency of radiolabelled red blood cells can be determined by gamma counting radioactivity in aliquots of the blood before and after centrifugation of the samples at 2000g for 20 min. The pre-centrifugation count then represents the total activity, whereas the remaining pellet after centrifugation and subsequent removal of the supernatant is a measure of cell bound activity. The total percentage of bound radioactivity can be calculated as:

$$\%\ \text{bound activity} = \frac{\text{activity in the pellet}}{\text{activity before centrifugation of the sample}} \times 100 \qquad (5.1)$$

Alternatively, to determine the labelling efficiency of the in vitro labelled red blood cells, the following procedure can be conducted:

(1) Remove the supernatant after the labelled blood has been centrifuged at 2000 rpm for 5 min;
(2) Measure the radioactivity of the red blood cells and supernatant and subtract the background from each measurement;
(3) The percentage binding can be calculated by dividing the counts (activity) of the red blood cells (RBC in Eq. (5.2)) by the sum of the counts (activity) of the red blood cells and supernatant.

$$\%\ \text{binding} = \frac{\text{activity of RBC}}{\text{activity of (RBC} + \text{supernatant)}} \times 100 \qquad (5.2)$$

Washing the red blood cells should be considered when the labelling efficiency is less than 90% but greater than 75%. When red blood cells are labelled in vivo, the labelling efficiency can only be determined after administering the radionuclide, and thus no preventive steps to improve labelling efficiency can be taken. A 5 mL blood sample can be obtained 5–10 min after administering the radionuclide and centrifuged as described above to determine the labelling efficiency. Images of the thyroid can be obtained to exclude free pertechnetate owing to poor labelling efficiency. If perchlorate is administered to block thyroid uptake of pertechnetate, imaging the thyroid region cannot be used to evaluate for free pertechnetate.

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6. REQUIREMENTS FOR RADIOLABELLING AND QUALITY CONTROL OF PLATELETS

This section provides some basic rules for the labelling of platelets and presents some basic regulations for the quality of radiopharmaceuticals. The available pharmacopoeias (e.g. European Pharmacopoeia and United States Pharmacopoeia) were used as a source for these standards. This section deals with the basic quality requirements for radioisotopes used for platelet labelling purposes.

As for the other labelled blood cells, it is equally important to adhere to strict quality control procedures when labelling platelets and to ensure correct patient identification when in vitro labelling techniques are used. The radiolabelling of platelets is still the method of choice to assess their survival time in vivo. It can also be used to image abnormal (arterial and venous) vascular and thrombotic lesions.

6.1. CONSIDERATIONS IN THE USE OF RADIOISOTOPES AND CHELATORS

Optimal radiolabelling is characterized by:

— Low elution rate;
— Acceptable physical characteristics;
— Low radiation;
— Easy and cheap availability;
— No re-utilization;
— No cellular re-uptake.

6.1.1. Chromium-51

Chromium-51 used as sodium chromate has the disadvantage that it has a long half-life and does not allow camera imaging with a rather low labelling efficiency and high elution compared with indium complexes. However, no re-utilization occurs. The major disadvantage is the high blood volume required (200–400 mL) due to the low labelling efficiency — even in patients with normal platelet counts.

6.1.2. Indium-111 compounds

Among the compounds examined, only ^{111}In-oxine and ^{111}In-tropolone are commercially available and are currently in use. In contrast to chromium, ^{111}In uniformly labels all isolated cells and is thus reliable for platelet survival studies. Indium-111 does not penetrate the cellular membrane, therefore chelating agents are used in order to induce a lipid soluble complex for labelling. Indium-111 complexes are highly lypophilic. Once in the platelets, approximately two thirds is bound to plasma proteins of molecular weight above 30 kDa, but the other third remains membrane bound.

6.1.3. Others

Technetium-99m (such as HMPAO and oxine) does not allow concomitant lifespan calculation, although sensitivity and specificity for imaging are reported to be comparable**.** PET tracers have also been unsuccessful, since they do not allow the monitoring of kinetics and the half-life is too short to visualize abnormal deposition sites (see also Section 1.3.3).

6.1.4. Indium chelators

Oxine, the first indium chelator used, gained wide acceptance due to simple handling and general availability [6.1]. Since uptake by cells is not specific, separation, pure preparation of cells and an absence of plasma are all necessary. The poor solubility of oxine in aqueous solutions requires a small amount of organic solvent.

6.2. INDIRECT LABELLING OF PLATELETS

The tentative of using antibodies that are mainly directed at surface glycoproteins (namely Gp IIb-IIIa) has failed. The theoretical advantages of avoiding cellular separation, enhanced target to background ratio and eventually better imaging have been eliminated by interference with functional properties of the cells. This has resulted in cellular activation which in several studies is evidenced by lung uptake and retention indicating microthrombi formation [6.2]. Kinetics monitored in parallel has consequently been unreliable.

6.3. CONSIDERATIONS IN THE USE OF ANTICOAGULANTS

ACD-A is advantageous over other acid dextrose solutions. Note that changes in the anticoagulant formula — in particular the pH — may cause platelet activation.

6.4. CONSIDERATIONS IN THE USE OF WASHING MEDIA

All washing media should be sterile. Incubation should be done in a freshly prepared Tyrode buffer at pH6.2 or in ACD saline. A higher pH may also cause platelet damage and aggregation. Note that the pH of the buffer is of key importance and may change during prolonged storage periods.

6.5. CONSIDERATIONS IN THE USE OF STABILIZERS

Prostaglandins and nitric oxide (NO) have been used to stabilize cellular membrane via cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) elevation, respectively. The addition of anti-aggregatory prostaglandins (PGI_2 or PGE_1) becomes relevant when more platelets are handled inappropriately or activated ex vivo. Owing to in vitro stability, PGE_1 is used more than PGI_2.

NO is a toxic and aggressive gas, which rapidly reacts with oxygen and is immediately deactivated by haemoglobin. The addition of NO donors has therefore been attempted. A dose of 1–5 mg/mL NO does not have a significant influence on labelling efficiency and recovery per se. Yet, when used in combination with PGI_2, a significant additive benefit in maintaining viability may be achieved. This benefit is emphasized when the platelet population is more functionally damaged.

6.6. CONSIDERATIONS IN THE USE OF DISPOSABLES

In order to avoid platelet activation, blood for platelet labelling should be withdrawn without venous occlusion slowly and using a large diameter needle. Whenever the type of disposables is changed, the adherence of platelets on the surface needs to be determined in advance, before clinical use. Usually, the adhesion should be less than 10% and ideally less than 5%.

6.7. CONSIDERATIONS IN THE USE OF CENTRIFUGES

To avoid platelet damaging, it is preferable to separate platelet rich plasma by low speed centrifugation (e.g. 150g) for a longer period of time (e.g. 5 min) rather than to increase the speed and to shorten the centrifugation time. The latter is more difficult to control and may easily cause more extensive platelet activation or damage. It is mandatory to keep the temperature in the centrifuge constant. Higher temperatures may activate platelets.

6.8. QUALITY CONTROL

6.8.1. In vitro quality control

6.8.1.1. Technetium labelled platelets

Technetium-99m labelled HMPAO is the ^{99m}Tc complex which is mainly used. In general, ^{99m}Tc is preferred for imaging quality but has the disadvantages that the labelling efficiency is lower, the elution rate is approximately 5 times higher in comparison to ^{111}In complexes and concomitant assessment of platelet survival is not possible. A consensus methodology is described in Ref. [6.3].

6.8.1.2. Factors influencing labelling characteristics

Indium-111 complexes are the first choice for platelet labelling [6.4]. It is of key importance to keep the number of cells close to, or above, 1×10^9/mL [6.5]. Cellular density and incubation temperature are the key determinants for appropriate labelling. It has been shown that the cellular membrane lipid composition significantly affects labelling efficiency and recovery [6.4]. Hypobetalipoproteinemia, but not hyperalphalipoproteinemia, improves labelling efficiency. It needs to be considered that severe thrombopathic conditions, such as leukaemia, may decrease labelling efficiency dramatically, even below 20%. To achieve adequate labelling in thrombocytopenia, a larger volume of blood should be withdrawn to reach a total amount of at least 150 000/μL (10 mL of blood give 50 000 platelets), and the incubation volume should be reduced. Both result in a higher cellular density during labelling [6.6].

The LDL cholesterol concentration in plasma is more closely related to impaired labelling data compared with total cholesterol. A significant impairment of labelling can be seen when LDL cholesterol exceeds 1.6 g/L and total cholesterol 3 g/L. Labelling efficiency and cell recovery are inversely correlated to LDL cholesterol ($r = -0.89$) and with total cholesterol ($r = -0.88$);

recovery with r = −0.82 and r = −0.81, respectively. There is, however, no significant correlation to HDL cholesterol or triglycerides. This suggests that plasmatic environment has no significant influence on labelling parameters. Hypertriglyceridemia, however, changes optical density and makes it more difficult to isolate platelets. The elution of indium is usually less than 0.5% per hour: free indium is rapidly trapped by circulating transferrin. This is one of the reasons why the incubation of platelets for radiolabelling needs to be performed in an absolutely plasma (protein) free environment. The shorter the total ex vivo period, the better are the recovery results. It is possible to perform the labelling procedure within 45 min, and the total time is strongly recommended not to exceed 60 min. The available complexes oxine and tropolone show identical labelling behaviour when compared with other compounds attempted, such as mercaptopyridinoxine. Antiplatelet agents (anti-aggregatory compounds such as acetylsalicylic acid, sulphinpyrazone, ticlopidine and clopidogrel) having been in contact with platelets in advance do not affect in vitro labelling behaviour. However, they may affect in vivo kinetics by making platelets less susceptible to adhere to lesion sites, which may subsequently result in less local trapping and negative imaging of (older) arterial or venous thrombosis or less active processes.

There is no in vitro parameter available to predict the late in vivo functional behaviour of the population of radiolabelled platelets. Platelet function testing requires a long time for the radiolabelled aliquot of cells to be reinjected, resulting in further functional impairment or damage of the latter. In the training period, however, it is strongly recommended to perform such tests to learn to keep the activation of platelets as small as possible. Platelet viability can be assessed in vitro using the nephelometric adenosine diphosphate (ADP) platelet aggregation test. When applied to labelled platelets, the results of this method will be hampered by the reduction of pH caused by ACD-A, so the platelet sample used needs to be washed and resuspended in plasma with normal pH. Running non-ACD-A treated samples (e.g. sodium citrate) simultaneously has the disadvantage of being a different probe and therefore hardly comparable. Other possibilities, although intrinsically limited to research fields, are the use of fluorescent antibodies against activated platelets or visualizing the platelets with a scanning electron microscope looking for morphological abnormalities due to activation or damage.

6.8.2. In vivo quality control

To determine the recovery 1–2 h after reinjection is the only reliable method to gain information on the functional stage of platelets. Recovery is normally in the range of around 20% below labelling efficiency. The shorter the ex vivo period of the platelets, the better is the recovery. The calculation of recovery

requires a blood sample drawn at the respective interval after reinjection of the labelled platelets and an estimate of the blood volume:

$$\text{recovery}\,(\%) = \frac{\text{blood activity per mL} \times \text{blood volume}}{\text{injected dose}} \times 100 \qquad (6.1)$$

Normal values are in the range of 55–72%, due to the early pooling of approximately one third of the platelets in the liver or spleen [6.7]. Impaired platelet function (e.g. cirrhosis and haematological disorders) may diminish the recovery.

Another index of platelet damage is an excessive liver uptake that may be assessed performing a dynamic acquisition during the injection, with liver and heart in the field of view. The time activity curves, obtained by drawing a region of interest (ROI) over both organs, need to show an almost parallel exponential decrease [6.8].

Platelet survival is determined by measuring the disappearance rate of radiolabelled platelets from circulation. At least three blood samples on the first day of reinjection of radiolabelled cells and at least one blood sample per day for a maximum of 7 days are required. For calculation, 1–2 mL blood samples are needed to isolate platelets (pelleting) and count radioactivity. The exact time of blood withdrawal needs to be given. Indium-111 platelets in healthy people disappear from circulation following a linear function. Abnormal trapping of platelets leads to a premature removal of the cells from circulation and a shortening of survival time. For the calculation of survival, a variety of mathematical models are available. The multiple hit, the weighted mean and the Dornhost models are recommended by the International Committee for Standardization in Hematology [6.9].

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7. CLINICAL USE OF RADIOLABELLED CELLS

7.1. RADIOLABELLED WHITE BLOOD CELLS

Radiolabelled white blood cells have been used in a variety of different clinical situations. However, owing to the variety of techniques employed and the limited number of patients included in published studies, it is difficult to draw evidence based statistical conclusions on the clinical relevance for most applications.

The results for the most important clinical indications are summarized in this section. Several reviews are available for an advanced and more comprehensive reading on the topic. Among the many reviews in the field, four useful papers have been published with meta-analysis of data collected between 1985 and 2005 on the clinical use of radiolabelled white blood cells compared with other available diagnostic techniques [7.1–7.4].

7.1.1. Osteomyelitis and spondylodiscitis

Osteomyelitis is an infection of the bone and medullary tissue due to the presence of aerobic or anaerobic microorganisms, viruses and fungi. It can represent a complication of a systemic infection or can be the manifestation of an infectious process that is situated primarily in the bone or surrounding tissue.

The most frequent origin is haematogenic, but the microorganisms can also reach the site of infection directly (exposed fractures and surgical procedures) or simply per contact. The haematogenic osteomyelitis is often caused by gram positive microorganisms, whereas fungi and mycobacteria frequently produce direct and chronic infections. Patients predisposed to osteomyelitis include those who are immunocompromised, have undergone radiotherapy, have diabetes mellitus or are suffering from a drug addiction.

Osteomyelitis can be classified as acute, sub-acute and chronic, according to the type of onset and clinical course. Patients with acute osteomyelitis usually present with fever, leukocytosis, elevation of infection parameters in the blood and pain (mild or intense) in the affected region. Rubor and swelling may also be present.

The diagnosis of osteomyelitis can be challenging, and radionuclide imaging procedures are routinely performed as part of the diagnostic workup. Bone scintigraphy is extremely sensitive, ubiquitously available, relatively inexpensive and rapidly completed. The accuracy of the test in unviolated bone exceeds 90% [7.5]. Many, if not most, patients referred for radionuclide evaluation of osteomyelitis, however, present with pre-existing conditions,

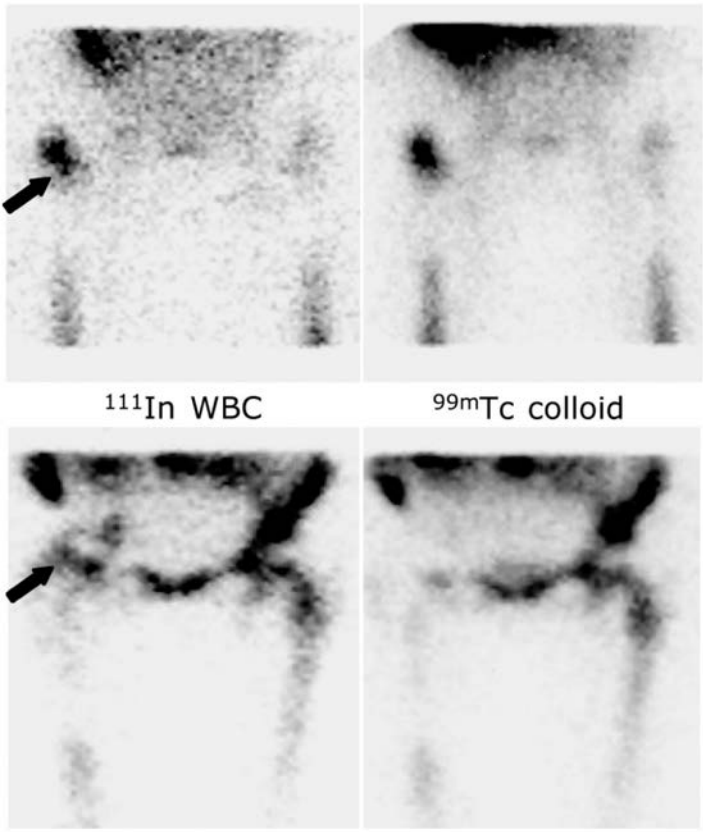
including fractures, orthopaedic hardware, pedal ulcers or neuropathic joints. These conditions adversely affect the specificity of the bone scan, necessitating the performance of additional imaging studies to differentiate infection from other causes of increased bone mineral turnover. Currently, labelled leukocyte imaging is the radionuclide procedure of choice for diagnosing osteomyelitis. In diabetic foot infections, the sensitivity of the test for diagnosing pedal osteomyelitis, using ^{111}In labelled leukocytes, is in the range of 72–100% and the specificity is in the range of 67–100%. The reported sensitivities and specificities of ^{99m}Tc-HMPAO labelled HMPAO leukocyte imaging for diagnosing diabetic pedal osteomyelitis are in the ranges of 86–93% and 80–98%, respectively [7.6].

In order to maximize the accuracy of labelled leukocyte imaging, the test often needs to be performed in combination with bone marrow imaging. The reason for this is that although leukocytes do not usually accumulate at sites of increased bone mineral turnover in the absence of infection, these cells do accumulate in the bone marrow. The normal distribution of haematopoietically active bone marrow is variable, and it may not be possible to determine whether a focus of activity on a labelled leukocyte image represents infection or atypically located — but otherwise normal — bone marrow [7.7]. This distinction can be made easily and accurately by performing bone marrow imaging using ^{99m}Tc colloids or ^{99m}Tc-albumin nanocolloids. Both labelled leukocytes and the sulphur colloids accumulate in the bone marrow; leukocytes also accumulate in infection, while the sulphur colloids do not (see Figs 7.1 and 7.2). The combined study is positive for infection when activity is present in the labelled leukocyte image without corresponding activity on the colloid bone marrow image. The overall accuracy of combined leukocyte/marrow imaging is approximately 90%. Combined leukocyte/marrow imaging is especially useful in the evaluation of prosthetic joint and neuropathic joint infections [7.8–7.11].

It is important to note that in contrast to other sites in the skeleton, labelled leukocyte imaging is not useful for detecting spinal osteomyelitis or spondylodiscitis (infection of two adjacent vertebral bodies and their intervertebral disk). Although increased uptake is virtually diagnostic of the disease, 50% or more of all cases present as areas of decreased, or absent, activity. Photopenia is not specific for vertebral osteomyelitis and is associated with a variety of non-infectious conditions including tumour, infarction and previously treated osteomyelitis [7.12].

7.1.2. Joint prosthesis and other orthopedic hardware

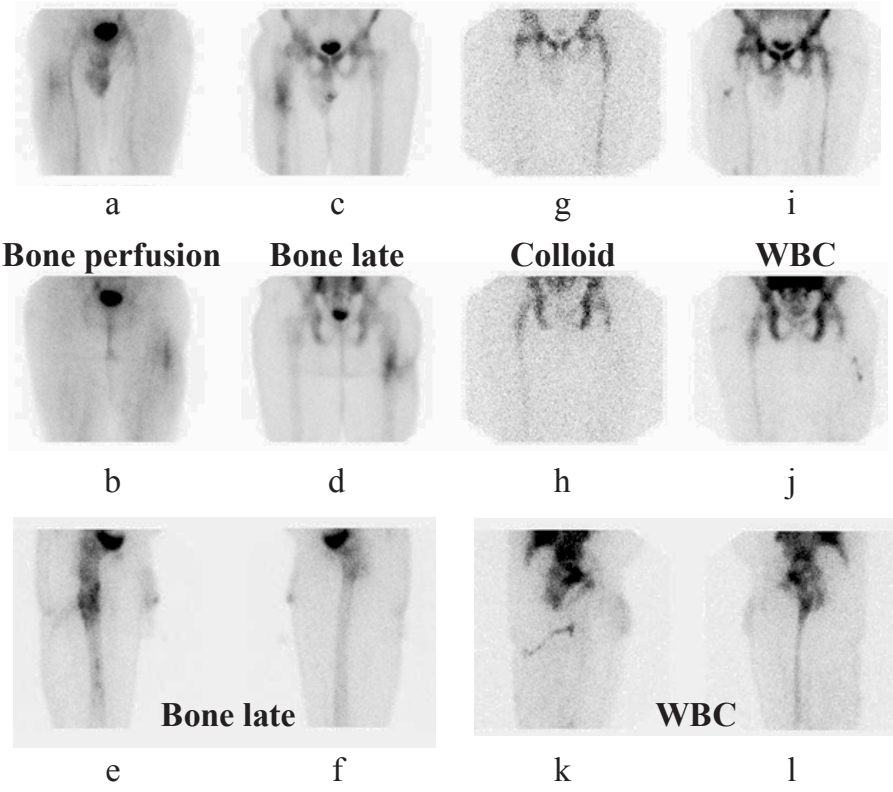
More than a million hip replacements are performed each year worldwide, and the number of other artificial joints (knees and elbows) inserted is also rising. Some complications of joint replacement surgery, such as dislocation and fracture,



Note: The images show the use of colloid scanned for differentiating infection from bone marrow activity in two patients. Both patients were scanned on suspicion of a hip infection. Patient with a matching colloid scan to ^{111}In labelled white blood cell (WBC) scan indicating absence of infection (*top row*). Patient with a mismatch between colloid scan and ^{111}In labelled white blood cell scan indicating the presence of infection (*bottom row*).

FIG. 7.1. The use of colloid scanned for differentiating infection from bone marrow activity (courtesy of C. Palestro, Long Island Hospital, New York).

are readily diagnosed and treated. With increasing numbers of implantations, infections and loosening of the prostheses have become more common. The risk of infection is greatest during the first two years following implantation. The duration and extensiveness of surgery is an important determinant of infection. The patient's age and immune status are also critical to the successful outcome of the implantation. Differentiating infection from aseptic loosening is difficult because the clinical presentation and the histopathological changes in both cases are remarkably similar. This differentiating is extremely important because

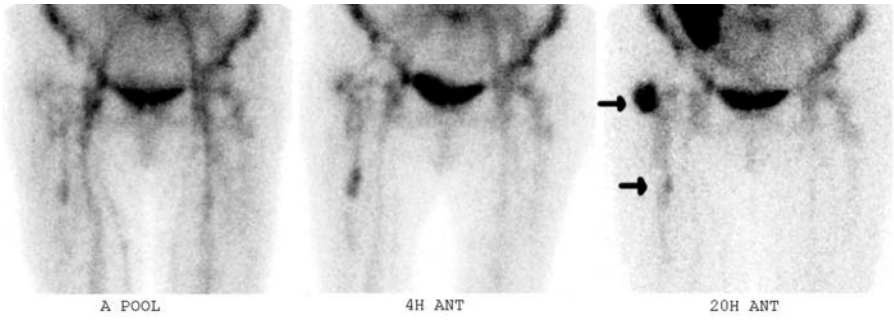


Note: Early (a: anterior, b: posterior) and delayed (c: anterior, d: posterior, e: right lateral, f: left lateral) bone scan showed abnormal uptake in the superior part of the femur. No abnormalities on colloid scan (g: anterior, h: posterior). The ^{99m}Tc-HMPAO labelled leukocyte study (i: anterior, j: posterior, k: right lateral, l: left lateral) demonstrates an increasing uptake in soft tissues of the right thigh, with no evidence of bone involvement.

FIG. 7.2. A 75 year old male with a cutaneous fistula and a history of a right femoral fracture, treated surgically 7 months previously.

the treatment of these two complications is different. Non-specific markers of inflammation such as the erythrocyte sedimentation rate and C-reactive protein level may be elevated in both loosening and infection. Joint aspiration with gram stain and culture is considered the definitive diagnostic test. However, its sensitivity is variable (in the range of 28–92%) and specificity is more consistent (in the range of 92–100%).

Plain radiographs are neither sensitive nor specific, and cross-sectional imaging modalities, such as CT and magnetic resonance imaging (MRI) are limited by hardware induced artefacts. Radionuclide imaging, reflecting physiological



Note: Planar anterior images were acquired at 30 min, 4 h and 20 h post-injection of ^{111}In labelled white blood cells. Two sites of accumulation of labelled cells can be seen in the 4 h image. The upper site increases in intensity with time (infection), while the lower site decreases with time (inflammation). The images clearly show the importance of sequential image acquisition when using radiolabelled white blood cells.

FIG. 7.3. Sequential images of the hip in a patient with suspected infected prosthesis (courtesy of C. Palestro, Long Island Hospital, New York).

rather than anatomical changes, is not affected by metallic hardware and plays an important role in the diagnosis of prosthetic joint infection. The current imaging procedure of choice for evaluating suspected joint replacement infection is combined leukocyte and bone marrow imaging with an overall accuracy in the range of 88–98%. Although inflammation may be present in both the infected and aseptically loosened device, neutrophils, which are invariably present in infection, are usually absent in aseptic loosening. The success of labelled leukocyte imaging is highly dependent on the presence of a neutrophil response, and this critical histological difference between infection and aseptic loosening accounts for the high sensitivity and specificity of combined leukocyte/marrow imaging for diagnosing prosthetic joint infection (see Fig. 7.3).

7.1.3. Inflammatory bowel diseases

IBDs are a group of idiopathic, chronic disorders, of uncertain aetiology, which include Crohn's disease and ulcerative colitis. Crohn's disease is a transmural, aspecific, chronic inflammatory disease that more frequently affects the final section of the small bowel (ileum) and the colon, but it can be localized in every region of the bowel with systemic pathological conditions. The incidence varies across countries, with the highest incidence in caucasians and with the highest peak at a young adult age, between 15 and 25 years (male:female ratio is 1:2). The precise aetiology is still unknown, but a complex interaction of

environmental, genetic, immunoregulatory and inflammatory factors are believed to play a role in the development of Crohn's disease (and also ulcerative colitis).

The disease is pathologically characterized by the presence of architectural distortion with transmural or superficial patchy granulomatous infiltration and the presence of acute inflammatory cells. The onset of disease is generally accompanied by diarrhoea, abdominal pain, the presence of blood and mucus in the faeces, weight loss and fever. These symptoms may be variably present, and there are also periods of complete well being. The disease may be complicated by perforations of the bowel wall, with abscess development, fistula and bowel bleedings.

Ulcerative colitis is a chronic inflammatory disease of the colon, restricted to the mucous and submucous wall of the colon. The disease can be limited to the rectal region (proctitis) or can extend to the sigmoid region (proctosigmoiditis) up to the left flexure of the colon (colitis) or even the right flexure (subtotal colitis). The peak incidence is between 15 and 30 years and between 50 and 70 years (male:female ratio is 1:3). Pathologically, the disease is characterized by diffuse mucosal inflammation and the presence of acute inflammatory cells. The symptoms are similar to Crohn's disease. Complications are perforations, fistula and abscess formation. In cases of large ulcerative processes, the toxic damage to the muscle tissue can induce the loss of neuromuscular function, with progressive dilatation of the colon (toxic megacolon).

In IBD, CT may be helpful to evaluate the presence of extramural complications such as abscesses, fistula and perforations. Scintigraphic imaging enables additional information to be obtained regarding the activity of the disease. Labelled white blood cell imaging is a useful adjunct to conventional diagnostic methods. It can be used as a screening test to identify patients who need further investigation. Labelled white blood cell imaging can also be used to monitor response to treatment, detect recurrent disease and determine the presence of active disease in patients whose physical presentation and laboratory test results are discordant. Although early studies were performed with ^{111}In labelled white blood cells, it is now agreed that ^{99m}Tc labelled white blood cells should be used. Imaging at multiple time points and single photon emission computed tomography (SPECT) maximize the sensitivity of the test [7.13]. There are, however, some limitations to labelled white blood cell imaging in IBD. The test cannot define anatomical changes such as strictures, which are best delineated with endoscopy and contrast radiography. It is less sensitive for upper, than for lower, gastrointestinal tract disease. The sensitivity of the test may also be affected adversely by corticosteroid administration [7.13].

7.1.4. Fever of unknown origin

FUO is defined as a body temperature higher than 38.3°C in more than one occasion during 2 weeks (or 3 days in the case of hospitalized patients without determining the cause). The most common causes of FUO are infections, malignancies, autoimmune diseases and collagen vascular diseases. In a significant number of cases (30%), the origin remains unknown despite medical investigation. Diagnosis is often made with the help of:

— Good anamnestic investigation (with special attention to job activity, recent travelling and drug abuse);
— Physical examination (searching for skin eruptions or lesions, lymphoadenopathy and hepatosplenomegaly);
— Blood tests (whole blood count, inflammatory markers and immune system profile);
— Microbiological cultural exams;
— Virus exams;
— Mantoux test (for tuberculosis).

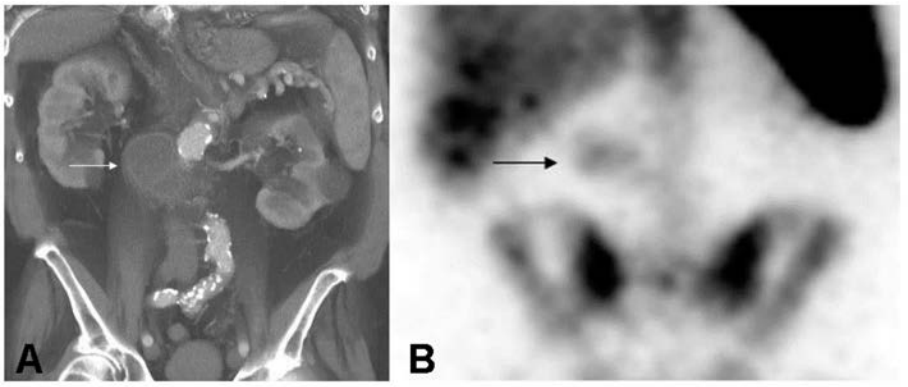
Radiological imaging modalities, such as ultrasound, CT and MRI, may be helpful for diagnosing FUO. In the majority of cases, however, the origin of the FUO remains uncertain.

Scintigraphic imaging with labelled white blood cell and PET imaging with FDG are accurate imaging modalities to evaluate patients with FUO. White blood cell imaging can yield useful information about the infection. A negative study virtually excludes infection or inflammation as the cause of the fever [7.6, 7.14]. It is accepted that when patients with FUO have a low probability of infection (low erthrocyte sedimentation rate, white blood cell count and C-reactive protein levels), an FDG–PET scan should be performed first. If patients exhibit a high probability for infection, they should first perform a white blood cell scan (see Fig. 7.4).

7.1.5. Soft tissue infections

7.1.5.1. Postoperative infections

Radionuclide imaging is a useful adjunct to morphologic imaging and can facilitate the differentiation of abscesses from other fluid collections, tumour and normal postoperative changes. Gallium-67 citrate can detect intra-abdominal infections. However, the presence of large bowel activity which can obscure foci of infection in addition to the need to wait 48 h or more between injection and



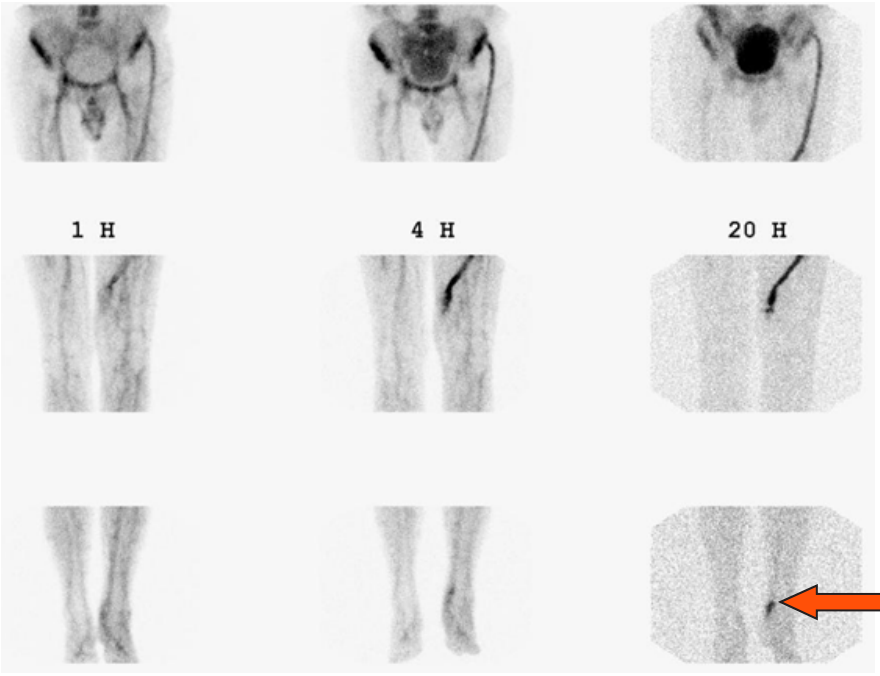
Note: A. Coronal CT image demonstrates a right sided retroperitoneal fluid collection (arrow). B. Anterior maximum intensity projection (MIP) image from an ^{111}In labelled leukocyte study performed around 24 h after demonstrates abnormal labelled leukocyte uptake (arrow) in the fluid collection. Staph aureus was cultured from the purulent drainage.

FIG. 7.4. Abdominal abscess (courtesy of C. Palestro, Long Island Hospital, New York).

imaging are the main disadvantages. Gallium-67 also accumulates in infections and tumours as well as in normal healing surgical incisions. Labelled white blood cells, in contrast, rarely accumulate in uninfected neoplasms and do not, with a few exceptions, accumulate in normally healing surgical incisions. Consequently, white blood cell imaging is the preferred radionuclide test for the evaluation of postoperative infections [7.15–7.17].

7.1.5.2. Cardiovascular system infections

Echocardiography is readily available and accurately diagnoses bacterial endocarditis. Radionuclide imaging methods play a very limited role in the diagnostic workup of this entity. Echocardiography is less sensitive, however, in the detection of myocardial abscesses. White blood cell imaging accurately detects myocardial abscesses in patients with infective endocarditis [7.18]. Prosthetic vascular graft infections, though uncommon, are very serious complications of vascular surgery. Imaging studies are routinely used to confirm or to exclude a vascular graft infection. CT is usually the initial imaging modality used. White blood cell imaging with either ^{99m}Tc or ^{111}In is a useful complement (see Fig. 7.5). The sensitivity of white blood cell imaging for diagnosing prosthetic vascular graft infection is in the range of 58–100%, although in the majority of papers it has been reported to be higher than 90%. Long duration of the symptoms or pretreatment with antibiotics does not adversely affect the study results. The specificity of white blood cell imaging is more variable and is the



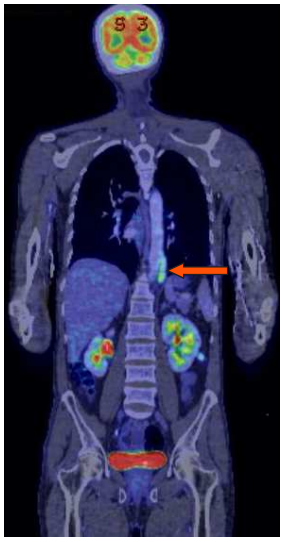
Note: The ^{99m}Tc-HMPAO labelled leukocyte study demonstrates abnormal uptake in the left femur, associated with a second image in the left medial malleolus, corresponding to a septic embolus (arrow).

FIG. 7.5. Image of a 60 year old male with an infected left femoral vascular graft implanted three months previously.

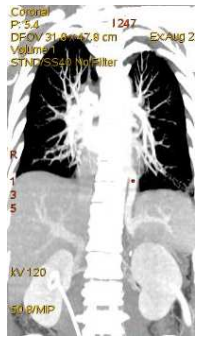
range of 53–100%. Causes of false positive results include perigraftic haematoma, bleeding, graft thrombosis, pseudo-aneurysms and graft endothelialization, which occurs within one to two weeks after placement [7.19–7.25]. FDG–PET/CT is a valid alternative for imaging vascular graft infections (see Fig. 7.6).

7.1.5.3. Pulmonary infections

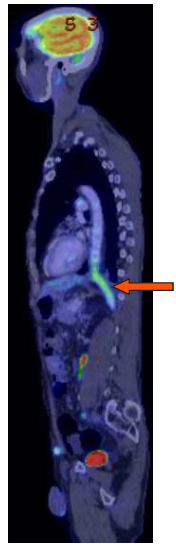
Although pulmonary uptake of labelled leukocytes is a normal physiologic event during the first few hours after injection, after 24 h such uptake is abnormal. Focal pulmonary uptake that is segmental or lobar in appearance is usually associated with bacterial pneumonia. This pattern is also seen in patients with cystic fibrosis and is due to white blood cell accumulation in pooled secretions in bronchiectatic regions of the lungs. Non-segmental focal pulmonary uptake is caused by technical problems during labelling or reinfusion and is not usually associated with infection.



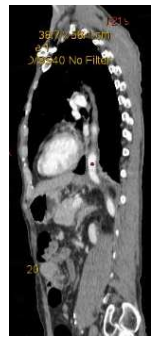
Fused FDG–PET/CT coronal image



CT coronal image (vascular enhancement)



Fused FDG–PET/CT sagittal image



CT sagittal image

Note: The FDG–PET/CT study demonstrates abnormal uptake in the aortic graft (arrow).

FIG. 7.6. Image of a 58 year old male with an infected aorto bifemoral vascular graft implanted one year previously.

Diffuse pulmonary activity on images obtained more than 4 h after reinjection of labelled cells is associated with opportunistic infections, radiation pneumonitis, pulmonary drug toxicity and adult respiratory distress syndrome. Diffuse pulmonary activity is also seen in septic patients with normal chest X rays who have no clinical evidence of respiratory tract inflammation or infection. Circulating neutrophils, activated by cytokines, pool in the pulmonary circulation because it is more difficult for them to manoeuvre through the pulmonary circulation. Cytokines presumably also activate pulmonary vascular endothelial cells, causing increased adherence of leukocytes to the cell walls, further retarding their transit through the pulmonary vasculature. Finally, it is important to note that while diffuse pulmonary activity on labelled white blood cell images is associated with numerous conditions, it is almost never seen in bacterial pneumonia [7.26, 7.27].

7.1.6. Other applications of radiolabelled white blood cells

7.1.6.1. Central nervous system infections

The differential diagnosis of a contrast enhancing brain lesion identified in CT or MRI includes abscess, tumour, cerebrovascular accident and even multiple sclerosis. White blood cell scintigraphy provides valuable information about contrast enhancing brain lesions. A positive study indicates that the origin of the brain lesion is almost assuredly infectious; a negative result rules out infection with a high degree of certainty. Faint uptake in brain tumours has been observed, and false negative results in patients receiving high dose steroids have been reported [7.28–7.30].

7.1.6.2. Acquired immune deficiency syndrome

White blood cell imaging has a very limited role in the evaluation of AIDS related infections. It is not sensitive for detecting the opportunistic infections that so often involve the lungs and lymph nodes of these patients. These results are not surprising because most opportunistic infections do not incite a neutrophilic inflammatory response. The test is useful, however, in detecting colonic infections in HIV positive patients [7.31, 7.32].

7.2. RADIOLABELLED RED BLOOD CELLS

Radiolabelled red blood cells can be used for a number of imaging and non-imaging investigations. Non-imaging studies include evaluating red blood cell survival and sequestration (using ^{51}Cr labelled cells) in patients with suspected haemolytic anaemia to determine the site of haemolysis. Chromium labelled red blood cells can also be used for the investigation of patients with chronic iron deficiency anaemia, when gastrointestinal blood loss is suspected but cannot be proven by conventional means.

Technetium-99m labelled red blood cells are used for:

— Blood pool imaging, first pass and MUGA cardiac studies;
— Localizing the site of acute gastrointestinal bleeding or other bleeding sites;
— Venography and haemangioma detection.

Although red blood cells can be labelled with ^{111}In, which is particularly helpful in discovering intermittent gastrointestinal bleeding, this approach is rarely used in routine clinical practice.

Selective imaging of the spleen with denatured red blood cells to investigate reticuloendothelial function is superior to sulphur colloid scanning because with the latter, the spleen can be masked by an overlapping left hepatic lobe. This is a particular problem when CT scanning is not available. Denatured red blood cell spleen scanning can be used to visualise accessory splenic tissue or splenunculi postsplenectomy.

7.2.1. Ventricular function

Ventricular function can be determined using the gated equilibrium radionuclide ventriculography technique. For this procedure, the patient's red blood cells are radiolabelled with ^{99m}Tc, and images are obtained with the patient connected to an electrocardiograph (ECG gating). Data are collected from several hundred cardiac cycles to generate an image set of the beating heart that is presented as a single, composite cardiac cycle. The method can be used to assess:

— Regional and global wall motion;
— Cardiac chamber size and morphology;
— Ventricular systolic and diastolic function, including left and right ventricular ejection fractions.

Studies may be acquired at rest, during exercise or after either pharmacologic or mechanical interventions [7.33, 7.34].

According to the guidelines of the Society of Nuclear Medicine and Molecular Imaging, common clinical settings in which radionuclide ventriculography may be useful include:

(a) Known or suspected coronary artery disease:
  (i) Without myocardial infarction;
  (ii) Remote myocardial infarction;
  (iii) Acute myocardial infarction (however, these patients usually should not undergo exercise stress in the first 48 h).
(b) Help in distinguishing systolic from diastolic causes of congestive heart failure in patients with known or suspected congestive heart failure.
(c) Evaluation of cardiac function in patients undergoing chemotherapy.
(d) Assessment of ventricular function in patients with valvular heart disease.

A radionuclide ventriculogram may be used in the conditions listed above for determining long term prognosis, assessing short term risk (e.g. preoperative evaluation), and monitoring response to surgery or other therapeutic interventions [7.35]. In several institutions, the determination of ventricular

function by radionuclide techniques has been replaced by echocardiography. For more details about procedure and elaboration/interpretation criteria for studying ventricular function, please refer to specific reviews and guidelines published in this field [7.36].

7.2.2. Gastrointestinal bleeding detection

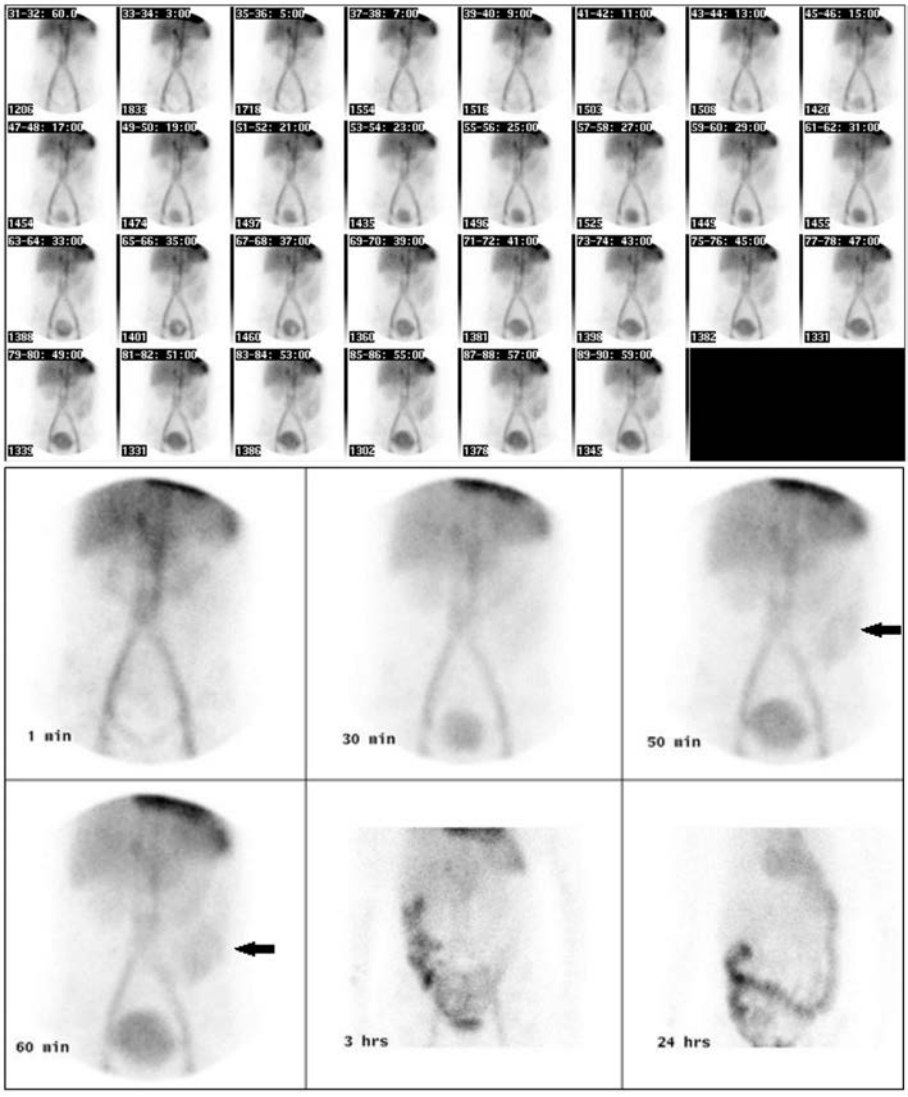
Gastrointestinal bleeding scintigraphy using ^{99m}Tc labelled red blood cells is performed in patients suspected of active gastrointestinal bleeding. Sites of active bleeding are identified by the accumulation and movement of labelled red blood cells within the bowel lumen. Since activity within the lumen of the bowel can move antegrade and retrograde, frequent images (one image every 60 s in either a dynamic or static mode) will increase the accuracy of localizing the bleeding site. Scintigraphy with labelled red blood cells is complementary to endoscopy and angiography because it permits continuous monitoring over several hours.

In patients suspected of low grade bleeding, usually presenting with iron deficiency anaemia without any other proof for gastrointestinal bleeding, red blood cell labelling with ^{51}Cr can be performed and stools collected over a period of three to four weeks after administering the ^{51}Cr labelled cells. If there is evidence for blood loss in the stool of >50mL/d, an imaging study with ^{99m}Tc labelled red blood cells can be performed to localize the bleeding site [7.37] (see Figs 7.7 and 7.8).

7.2.3. Spleen imaging

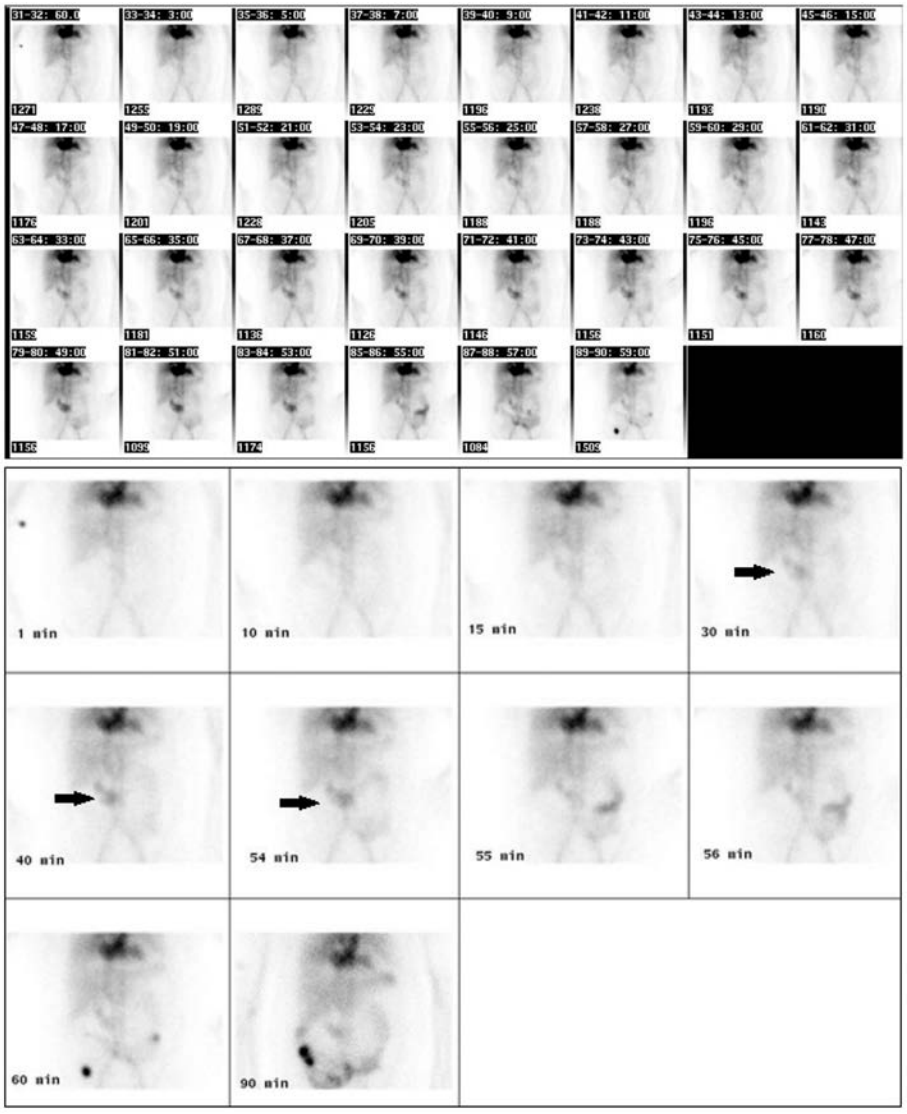
The reticuloendothelial function of the spleen can be investigated by imaging the trapping of damaged erythrocytes, using the spleen's function known as culling or pitting. This is achieved with heat damaged denatured labelled erythrocytes that are removed almost exclusively by the spleen.

Technetium-99m labelled denatured red blood cells are used to diagnose congenital asplenia, polysplenia, rudimentary spleens or acquired spleen pathologies (e.g. after trauma or in the investigation for splenunculi). Sometimes, imaging with denatured labelled erythrocytes is used to determine whether a splenectomy, splenic embolization or radiation treatment have been successful, particularly in patients with thalassaemia and in recurrence of idiopathic thrombocytopenic purpura after splenectomy or splenic ablation [7.38–7.40] (see Fig. 7.9).



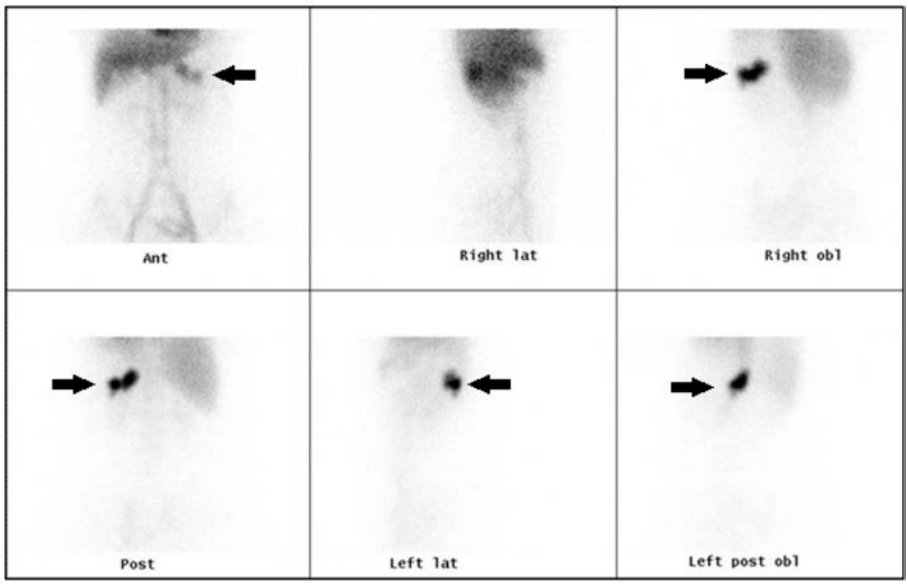
Note: Patient with Glanzman disease with dysfunctional platelets and suspicion of gastrointestinal bleeding. A red blood cell study clearly shows a bleeding site in the small bowel in the left iliac fossa (arrows), with gradual movement of the labelled cells to the large intestine in the later images. Selective angiography of the superior mesenteric artery confirmed a capillary blush in the vasa recta of the jejunum.

FIG. 7.7. Dynamic (top) and static (bottom) views of a gastrointestinal bleeding scintigram (courtesy of A. Ellmann, Stellenbosch University and Tygerberg Hospital, Cape Town).



Note: Patient presented with melaena and a low haemoglobin level. The labelled red blood cell study shows active bleeding in the small bowel (arrows), with a fast movement to the large intestine. A laparotomy followed and a bleeding duodenal ulcer was found.

FIG. 7.8. Dynamic (top) and static (bottom) views of a gastrointestinal bleeding scintigram (courtesy of A. Ellmann, Stellenbosch University and Tygerberg Hospital, Cape Town).



Note: Patient known with idiopathic thrombocytopenic purpura with splenectomy six years before and low platelet count despite treatment. Red blood cell imaging was performed after administering denaturated ^{99m}Tc labelled red blood cells because residual splenic tissue was considered. An increased concentration of red blood cells can be seen in the splenic region (arrows), indicating residual splenic tissue then confirmed by ultrasound.

FIG. 7.9. Spleen imaging with radiolabelled heat damaged red blood cells (courtesy of A. Ellmann, Stellenbosch University and Tygerberg Hospital, Cape Town).

7.2.4. Red blood cell volume determination

Blood volume measurements can be performed to determine the further management of patients with a raised haemoglobin or haematocrit [7.41]. Chromium-51 was first used to label red blood cells [7.42] and is still seen as the gold standard for red blood cell volume determination. Technetium-99m labelled red blood cells can also be used to determine red blood cell volume [7.43]. The accuracy of the ^{99m}Tc labelled red blood cells compares well with the ^{51}Cr technique. Iodine-125 human serum albumin is often used concomitantly for the determination of plasma volume, but a description of this procedure is beyond the scope of this publication.

7.2.5. Other applications of radiolabelled red blood cells

Patients with haemolytic anaemia of different aetiologies can be investigated using the ^{51}Cr red blood cell survival and sequestration technique [7.44].

7.3. RADIOLABELLED PLATELETS

Radiolabelled platelets have been mainly used in two different clinical settings: the identification of pathological areas of deposition; and the study of the sites of destruction in patients with thrombocytopenia. In the former case, anticoagulants or drugs known to have an anti-aggregatory effect, such as non-steroidal anti-inflammatory drugs and dipyridamole, may interfere with platelet deposition at pathological sites.

7.3.1. Deep venous thrombosis

Scintigraphy provides the best results in cases in which the platelets are already radiolabelled at the onset of thrombosis. Positivity of images with radiolabelling after the event depends on the activity of the process and the amount of platelets locally trapped [7.45]. Venous thrombosis usually shows up during the initial 10 days after the event or even later, if significant platelet apposition occurs. Antiplatelet substances may reduce the residence time of platelets and consequently the target to background ratios. A thrombus needs to contain at least 0.1% of total injected cells to significantly show up by scintigraphy. A same day gamma camera imaging after 4–6 h and a 24 h image are recommended. The later and the less lesion activity to show up in gamma camera imaging, the less is the activity of the process. This technique is nevertheless clearly less ready to use and more expensive than Doppler ultrasound or phlebography. It may be used, however, as an alternative if other techniques are contraindicated or unsuccessful [7.46].

7.3.2. Intracardiac thrombi

Scintigraphy provides useful information on the presence of intracardiac thrombi that may not be available from any other tests [7.47]. It may identify a thrombus even before it reaches sufficient size to be detected by echocardiography simply by its activity. Imaging up to 72 h is sometimes necessary to visualize the thrombus.

7.3.3. Haematologic diseases

Assessment of platelet survival and identification of the site of platelet destruction is a cornerstone of the therapeutic strategy in patients with idiopathic thrombocytopenic purpura, allowing the clinician to select patients for splenectomy with high accuracy [7.48] (see also Section 8.2.3).

7.3.4. Other applications of radiolabelled platelets

Platelet imaging has been successfully used to determine the activity of various antiplatelet drugs, such as acetylsalicylic acid, dipyridamole, sulphinpyrazone, prostaglandins, calcium channel blockers and angiotensin converting enzyme inhibitors, among others [7.49]. Surprisingly, when the platelet treatment is stopped, a decreased deposition of platelets at lesion sites persists for weeks or even months. Normal local platelet uptake at lesion sites is inversely related to platelet survival, especially when the number and extent of the lesions are increasing.

In immune thrombocytopenic purpura, scintigraphic imaging enables the platelet trapping and destruction in the spleen (spleen/liver ratio) to be assessed quantitatively [7.50]. This procedure is widely used to support the decision for a splenectomy.

Abnormal platelet deposition on synthetic material may result in thrombosis, thromboembolism or graft failure. Clinical studies in humans have revealed that platelet graft interaction, thrombotic activity, the efficacy of antithrombotic therapy and the occlusion rate can all be quantitatively assessed.

During acute vascular rejection of grafts, platelet deposition is an early event and has been successfully used for diagnosis [7.51]. However, the introduction of newer immunosuppressive drugs dramatically decreased the rate of acute vascular rejection episodes and also significantly reduced the clinical value of this indication.

It needs to be taken into consideration that any significant contamination with other blood cells may non-specifically demonstrate a variety of other clinical conditions. In particular, the presence of haematoma or varicose veins needs to be considered, since it may significantly influence platelet decay and distribution [7.52]. Thus, a whole body image is always recommended; a positive platelet image, however, may also be obtained at inflammatory lesion sites, as well as around various tumours.

REFERENCES TO SECTION 7

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8. REQUIREMENTS FOR CLINICAL PROCEDURES

Image acquisition protocols and image interpretation criteria may vary from centre to centre and depend on the experience of physicians and institutions as well as various other factors, such as the type of equipment available (single head, double head cameras, SPECT and SPECT/CT) in addition to the technique used and injected activity. It is therefore difficult to make general guidelines. This section is aimed at providing general recommendations and at explaining the advantages and disadvantages of different acquisition protocols in order to minimize interpretation problems.

8.1. PATIENT RELATED CONSIDERATIONS

8.1.1. Pre-appointment considerations

For all studies using labelled blood products, it is essential that measures are in place to identify the patient correctly to avoid administering labelled cells to the wrong person. As for nuclear medicine studies, pregnancy should be excluded in all female patients who are in their reproductive years. Patients who are breastfeeding need to be informed about the correct procedures to follow — for example, the period of interruption of breast-feeding, the importance of limiting the contact dose and the possibility of collecting some breast milk for use during the period in which the baby should not be breastfed [8.1–8.3]. It is advisable not to investigate female patients during menstrual bleeding, and it should be brought to their attention that sanitary towels or tampons may become radioactive and are therefore considered to be contaminated waste.

8.1.1.1. White blood cells

Patients should preferably be fasting at the time of sampling, although this is not strictly necessary. The possible interaction of high levels of cholesterol and glucose in the blood needs to be taken into account. A careful history needs to be obtained from the referring physician to ensure that the correct procedure will be applied (e.g. the use of ^{111}In or ^{99m}Tc labelled white blood cells, and the type and time of acquisition). The blood results of the patient need to be checked to assess whether infection parameters in the blood are elevated and whether enough white blood cells are available for labelling. Furthermore, the possible interference of some drugs and antibiotics needs to be evaluated. However, it is presently not possible to exclude a priori patients under antibiotic treatment,

since several authors described no interference between treatment and accuracy of a white blood cell scan [8.4]. Others advise repeating the scan after two weeks of therapy withdrawal in the case of doubtful scans [8.5].

8.1.1.2. Red blood cells

Aspects that should be considered when making appointments for labelled red blood cell studies will depend on the clinical indication for the study. Sufficient information needs to be obtained from the referring physician to ensure that the correct study is performed for the specific clinical indication and that the patient is informed and prepared according to the indication for the study.

No specific patient preparation is necessary for the majority of labelled red blood cell studies. When red blood cells are labelled in vivo for imaging studies, perchlorate may be given to block the uptake of free pertechnetate in the thyroid and stomach. Technetium-99m red blood cell labelling failed when associated with prior chocolate ingestion [8.6]. The labelling rate may be inhibited owing to the cacao components that modulate red blood cell and plasma oxidoreductive status, also acting on the red blood cell membrane permeability and plasticity. Twelve hours of limited consumption is adequate. In the case of studies to investigate bleeding sites, in vitro labelling or the in vivo modified technique are preferred to prevent free pertechnetate interfering with image interpretation.

With regard to spleen imaging with denatured red blood cells, recent splenic surgery should be recorded, as this can result in the activation of ectopic splenic tissue or reveal the presence of residual splenic tissue left behind. If the patient has had trauma in the left quadrant and thorax, images should include the thorax to exclude the possibility of a diaphragmatic rupture.

Careful attention should be paid to the medication the patient is currently taking, as several drugs are known to interfere with red blood cell labelling, such as heparin, methyldopa, hydralazine, propranolol and iodinated contrast media. Immunosuppresive drugs such as cyclosporine have been reported to decrease red blood cell labelling, as they may saturate the erythrocytic membrane [8.7]. However, it has been shown that there is no interference at therapeutic levels of cyclosporine [8.8, 8.9] (see Section 5.6 for more details).

8.1.1.3. Platelets

Peripheral platelet count, blood sedimentation rate, plasma cholesterol and triglyceride level should be known in advance. If peripheral platelet count is low, the volume of blood withdrawn needs to be increased. If the sedimentation rate is below 2–4 mm/h, blood dilution with citrate should be performed to obtain a sufficient number of platelets. Very high levels of cholesterol (>3 g/L) are

inversely correlated to labelling efficiency and platelet recovery. Similarly, high levels of plasma triglycerides (due to dyslipidaemia or postprandial sampling) may impair platelet separation.

8.1.2. Pre-injection considerations

When cells are labelled, universal precautions should be followed by the operator, as transmission of pathogens may occur. Blood samples should be treated with utmost care by the staff handling the patient's blood owing to the potential source of HIV/hepatitis contamination, parasitic infections or other microbiological infections. Care should be taken at all times, since patients may not be aware of their HIV/hepatitis status.

Careful attention should be paid to the medication the patient is taking, as several drugs are known to interfere with red blood cell labelling, as discussed in Sections 5.6 and 8.1.1.2.

Each nuclear medicine department needs to have specific measures in place to ensure that blood samples for cell labelling are identified in such a way that there is no risk of the blood sample being administered to the wrong patient (e.g. that two people always check before the labelled blood cells are administered). Labelling more than one blood sample at the same time in the same laminar flow hood should be avoided.

In paediatric patients with an indwelling intravenous line, in vitro labelling for red blood cells may be a better choice, as the same venous access can be used without the necessity to look for another peripheral vein for reinjection. If possible, a peripheral vein should be used in adults that is not used for other medication.

8.2. IMAGING PROTOCOLS

8.2.1. Imaging of white blood cells

For the image acquisition, a large field of view gamma camera with a low energy high resolution collimator is usually preferred (140 keV using a 15–20% window).

When ^{99m}Tc labelled white blood cells are used, early imaging of lungs for quality control is essential, as well as pelvis and abdomen, because normal bowel activity is seen in 20–30% of children at 1 h after injection and 2–6% of adults at 3–4 h. Lung images at 30 min are therefore suggested and whole body images at 3 h and 24 h. Images of the regions of interest (ROIs) also need to be obtained for at least 800 000 counts/large field of view or 5–10 min/view.

Another option is to perform early images at 30 min, followed by 6 h images (between 3 h and 24 h) that provide good information on the positivity/negativity of the lesions. In this case, delayed images (i.e. at 24 h) can be acquired only in the case of doubtful images at 6 h. This protocol, however, still needs validation. Alternatively to counting corrected images, time corrected images for isotope decay have been suggested (i.e. 200 s at 3 h and 2000 s at 24 h for ^{99m}Tc; 400 s at 3 h and 500 s at 24 h for ^{111}In). This method reduces operator interference in final image interpretation and an objective increase of activity or size with time in infected sites can be easily identified. This approach also makes quantitative analysis more accurate. If early and delayed images are acquired with the same numbers of counts, delayed images should be corrected for the radioisotope half-life. A limited study to evaluate a specific region of the body is acceptable in selected cases. SPECT or SPECT/CT images are mandatory in selected indications (e.g. endocarditis). SPECT or SPECT/CT scans at 3 h (or 6 h) are usually acquired with a 15–20 s/step protocol with a 64 × 64 matrix and with a 30–40 s/step protocol at 24 h.

In the case of abdominal infections and IBD, images can only be acquired at 30 min and 3 h after injection of ^{99m}Tc-HMPAO labelled white blood cells. This is because ^{99m}Tc-HMPAO is released by white blood cells with time, taken up by the liver and excreted via the bowel, thus producing false positive images at later time points. This problem does not occur in the case of ^{111}In labelled white blood cells, which are preferable for studying abdominal infections. For the same reason, when using ^{99m}Tc labelled white blood cells, vascular graft infections of abdominal vessels (aortoiliac grafts) should be imaged within 3 h from administering labelled cells. An early dynamic acquisition (one image every 5 s for 150 s) soon after the injection of labelled cells may help to map the vascular structures and to detect obstructions or aneurisms.

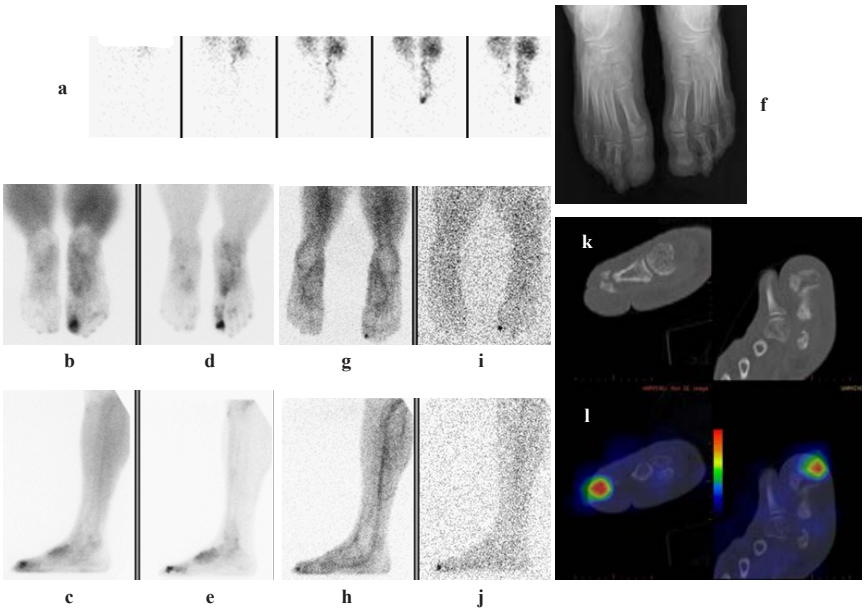
Accurate interpretation of labelled white blood cell scintigraphy requires knowledge of the normal and abnormal variants of white blood cell localizations. The diagnosis of an infection is made by comparing early and delayed images. The images are classified as:

(a) Negative if no uptake or a significant decrease in uptake from early to delayed images is present.
(b) Positive when uptake is seen in both early and delayed images which increases in time.
(c) Equivocal when the uptake in early and delayed images is the same or slightly decreasing.

After visual assessment, semiquantitative evaluation may also be performed. The purpose is to evaluate whether quantification of uptake could help to

differentiate infection from non-specific uptake. ROIs are drawn over the hottest region and copied to presumed normal reference tissue (e.g. anterior superior iliac crest, unaffected proximal, or distant or contralateral bone). The mean counts per pixel in these ROIs are recorded to calculate lesion to reference (L/R) ratios in both early and delayed images (L/R_{early} and L/R_{late}, respectively). When the L/R ratio increases with time ($L/R_{late} > L/R_{early}$), the scan is considered indicative of infection. When the L/R_{late} is similar or slightly decreased with respect to L/R_{early}, the examination is classified equivocal. When L/R_{late} is significantly decreased compared with L/R_{early}, the examination is classified negative for infection.

If SPECT/CT images are used, the delineation of the site of increased radiopharmaceutical uptake may be calculated by a 50% isocontour on a single transaxial slice with the hottest activity (lesion) and the reference tissue (e.g. anterior superior iliac crest). The same criteria as described above may be used for imaging classification (see Fig. 8.1).



Note: Three phase bone scan: dynamic (a: plantar view), perfusion (b: plantar view, c: lateral view) and static (d: plantar view, e: lateral view) phases showing uptake in the first toe of the right foot. Bone alterations are shown in the planar X ray image (f). Planar white blood cell images performed at 1 and 20 h after re-injection (g: earlier plantar view, h: earlier lateral view, i: later plantar view, j: later lateral view) and fused images (k: CT images, l: SPECT/CT images) confirmed the infection, located at the inter-phalangeal joint.

FIG. 8.1. A 78 old female with diabetic foot and osteomyelitis of the first toe of the right foot (evaluation after one month of antibiotics).

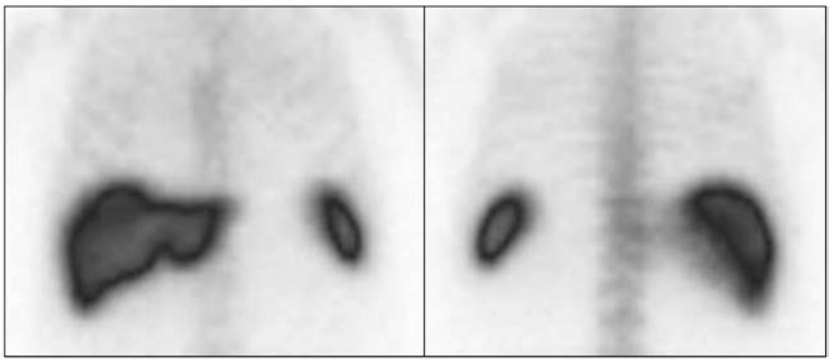
8.2.2. Imaging of red blood cells

Imaging protocols for studies using labelled red blood cells will be determined by the clinical indication for the study. As several procedures can be performed with labelled red blood cells, these will not be described in this publication. Readers are referred to the IAEA publication Nuclear Medicine Resources Manual [8.10] for specific clinical indications, patient preparation, procedures and sources of error.

8.2.3. Imaging of platelets

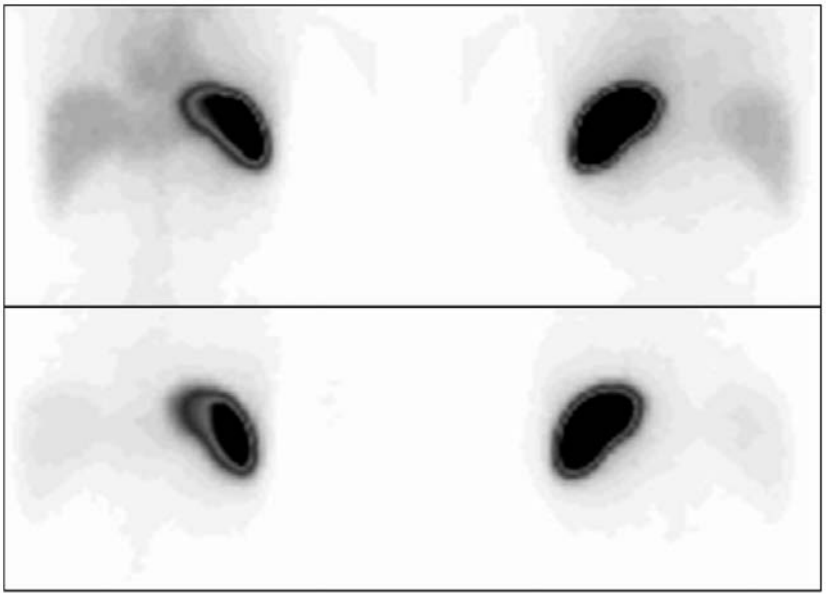
The timing of radiolabelled platelets (with indium compounds) imaging depends largely on the purpose of the study. Early imaging should be done at 60 min after injection to assess stable and normal distribution in the spleen and liver. For assessing abnormal platelet deposition in vessels which are suspected or known to have a thrombus or a plaque, usually two scans at 24 h and 48–72 h may be enough, targeted at the site involved. A whole body scan should always be performed so as not to overlook aneurysm or other abnormal accumulation. Of course, this is a field with limited and heterogeneous experiences. For studying sites of platelet destruction in patients with idiopathic thrombocytopenia or idiopathic thrombocytopenic purpura, at least two images of the upper abdomen, with both liver and spleen in the field of view, are required — the first a few hours (usually 2 h) after injection and the second 3–7 days after injection. Activity ratios between spleen and liver can be measured with the geometric mean of the counts. When the spleen/liver ratio exceeds 1.5, it can be considered as abnormal. However, sequestration is not based on activity ratios alone. The dynamics of the uptake, by calculating the counts over time, is even more important. Therefore, more images (each day for at least 7 days) are necessary. An excess of counts over time in the spleen is considered abnormal. It also needs to be taken into consideration that ^{99m}Tc-HMPAO labelled neutrophils migrate preferentially to the liver, whereas ^{99m}Tc-HMPAO labelled eosinophils migrate preferentially into the spleen. Therefore, a high liver uptake can be observed in neutrophilic patients with a very low number of eosinophils (<1%).

To calculate the platelet survival time, blood samples need to be withdrawn early (after 30 min and after 2–3 h), 24 h after the injection and each subsequent day for at least a week. Fixed time intervals are not necessary; it is better to advise people to provide the real time the samples were drawn (see Figs 8.2–8.4; see also Section 6.8.2).



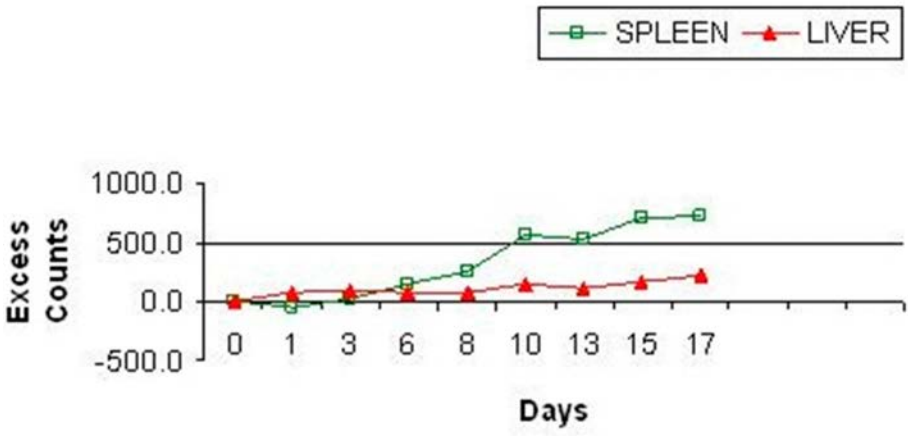
Note: Images obtained 3 days after reinjection of ^{111}In-oxine labelled autologous platelets in a patient without prevalent splenic destruction signs. The spleen/liver uptake ratio is 0.5 and therefore considered normal.

FIG. 8.2. Autoimmune idiopathic thrombocytopenia (courtesy of A. Ellmann, Stellenbosch University and Tygerberg Hospital, Cape Town).



Note: Images obtained 2 h (top row) and 3 days (bottom row) after reinjection of ^{111}In-oxine labelled autologous platelets in a patient with prevalent splenic destruction. The spleen/lever uptake ratio is 8 and therefore considered abnormal.

FIG. 8.3. Autoimmune idiopathic thrombocytopenia (courtesy of A. Ellmann, Stellenbosch University and Tygerberg Hospital, Cape Town).



Note: Increasing spleen/liver radioactivity ratio over time (up to 17 days after injection) with an excess of counts from day 6 in the spleen compared with the liver. This is a sign of abnormal platelet uptake in the spleen.

FIG. 8.4. Increasing spleen/liver radioactivity ratio over time (courtesy of A. Ellmann, Stellenbosch University and Tygerberg Hospital, Cape Town).

8.3. CONSIDERATIONS IN THE IMAGE INTERPRETATION OF WHITE BLOOD CELLS

8.3.1. Criteria for positivity

The criteria for positivity include any increase of uptake, or of the size of uptake, over time.

8.3.2. Qualitative analysis

Images need to be evaluated without too many operator modifications. Iliac bone uptake should be taken as the reference region whenever possible.

8.3.3. Semiquantitative analysis

In the case of doubt, it is suggested to calculate the *L*/*R* ratios in early and delayed images as described in Section 8.2.1. The anterior superior iliac crest is to be used as a reference region whenever possible.

8.3.4. Additional investigations based upon the imaging results

The fusion of white blood cell images with CT images of the part of the body suspected with infection or inflammation may be helpful for a more accurate localization of white blood cell uptake, particularly in differentiating soft tissue uptake from bone uptake (e.g. in diabetic foot). In diabetic foot, superficial and soft tissue infections may often show a decrease of activity with time between 3 h and 24 h images, but osteomyelitis always shows an increase of activity with time. SPECT/CT also helps in differential diagnosis. In Charcot foot, an additional scan with colloids for bone marrow imaging is mandatory to differentiate between expanded bone marrow (a common finding in Charcot foot) and osteomyelitis.

When using ^{111}In labelled white blood cell for IBD evaluation, stool collection and counting at an early and late time point may indicate the presence of radiolabelled cells migrating from infected mucosa to the lumen, an indirect sign of infected bowel. Planar acquisitions in the 'sitting' position — sitting the patient on the gamma camera, known as the tail on the detector (TOD) position — may better discriminate between rectal and bladder activity in the case of suspected rectosigmoidal extent of IBD.

In vascular grafts, oblique views may help to differentiate between uptake in the graft itself or in the surrounding tissue.

8.3.5. Pitfalls and artefacts

Regardless which tracer is used, uptake of labelled white blood cells depends on intact chemotaxis, the number and types of cell labelled, and the cellular component of a particular inflammatory response. The labelling of white blood cells, now a routine procedure, does not affect their chemotactic response. A total white blood cell count of at least 2000/μL is needed to obtain satisfactory images. In most clinical settings, a mixed leukocyte population is labelled. Hence, the majority of cells labelled are neutrophils, and the procedure is most useful for identifying neutrophil mediated inflammatory processes, such as bacterial infections. The procedure is less useful for those illnesses in which the predominant cellular response is not neutrophilic (i.e. opportunistic infections, tuberculosis and sarcoidosis). Although pulmonary uptake of labelled leukocytes is a normal physiologic event during the first few hours after injection, at 24 h after injection such pulmonary uptake is abnormal. Focal pulmonary uptake that is segmental or lobar in appearance is usually associated with infection. Non-segmental focal pulmonary uptake, however, is caused by technical problems during labelling or reinfusion and is generally not associated with infection.

Indium labelled leukocytes do not accumulate in a normal bowel. Bowel activity is always abnormal and is seen in antibiotic associated colitis, pseudomembranous colitis, infectious colitis, IBD, ischemic colitis and gastrointestinal bleeding.

Radiolabelled leukocytes do not accumulate in normally healing surgical wounds, so that the presence of such activity indicates infection. There are, however, certain exceptions. Granulating wounds that heal by secondary intention can appear as areas of intense activity on white blood cell images, even in the absence of infection. Examples include stomies (tracheostomies, ileostomies and feeding gastrostomies) and skin grafts. Vascular access lines, dialysis catheters and even lumbar punctures can all produce false positive results in the absence of appropriate clinical history.

8.4. CONSIDERATIONS IN THE IMAGE INTERPRETATION OF RED BLOOD CELLS

Interpretation of studies using labelled red blood cells will be determined by the clinical indication for the study. A detailed description of this is beyond the scope of this publication. Readers are referred to the IAEA publication Nuclear Medicine Resources Manual [8.10] for specific clinical indications, patient preparation, procedures, image interpretation and sources of error.

8.4.1. Qualitative analysis

Blood pool imaging for equilibrium studies requires a tracer that remains stable within the vascular compartment during data acquisition. Technetium-99m is the tracer most universally employed for the labelling of red blood cells with continuous ECG monitoring. Problems arise when the R-R interval on the ECG varies. Techniques are available to correct extrasystoles and sinus arrhythmia. If the heart rhythm is irregular, however, as in atrial fibrillation, this methodology is not valid [8.11].

8.4.2. Semiquantitative analysis

Technetium-99m labelled denatured red blood cells disappearance from circulation is useful in determining whether the anaemia is related to hypersplenism. Normal cell transit time in the spleen is 30–60 s; in splenomegaly, the mean transit time is prolonged.

The simplest way to measure the rate of disappearance in the bi-exponential curve is the half-life of the denatured red blood cells in the spleen. However,

this half-life should be used with caution because of overprojection of the liver. The clearance of denatured red blood cells depends on three processes:

(a) Splenic blood flow;
(b) Splenic denatured red blood cell extraction rate;
(c) Intrasplenic transit time of 'unextracted' denatured red blood cells that return to the circulation.

The percentage fall in denatured red blood cells between 8 and 28 min is therefore more precise [8.12].

8.5. CONSIDERATIONS IN THE IMAGE INTERPRETATION OF PLATELETS

8.5.1. Criteria for positivity

No clear cut definition concerning positivity exists. Usually, an uptake of more than 5% compared with blood background shows up clearly in camera imaging. It is, however, assumed that an uptake of more than 20% of counts per pixel — compared with the contralateral unaffected side — should be judged as significantly positive. However, this approach is not applicable in special conditions, such as after an amputation. Various attempts for calculations including blood and heart activity have not resulted in a clear definition.

8.5.2. Qualitative analysis

A pathological accumulation of radiolabelled platelets can be defined as a localized, presumably intravascular, 'hot spot', with a trend to increase its target to background ratio from early to late image.

8.5.3. Semiquantitative analysis

The insertion of ROIs over lesion sites assessing counts per pixel is the most widely used technique. More sophisticated techniques, such as blood pool labelling to subtract circulating blood, have been found to induce higher costs, prolonged duration of investigation and a higher radiation burden, but have not been found to result in improved clinical results.

In patients with thrombocytopenia, hypersplenism has been defined as a spleen/liver uptake ratio above 1.5 at both early and late images [8.13, 8.14]. Calculation of spleen/liver ratios requires ROIs to be inserted over the whole

organ. Platelets normally survive for 10 days in circulation. A shortened survival reflects functionally damaged cells and sites of abnormal trapping. For identification of the latter, whole body images should be performed and blood samples are suggested for a minimum of 7 consecutive days.

8.5.4. Additional investigations based upon the imaging results

In patients with thrombocytopenia, no additional investigation is required. Results of studies aimed at the identification of thrombi are usually compared with the conventional radiological workup.

8.5.5. Pitfalls and artefacts

In vitro activation of platelets during isolation and labelling may lead to artificial shortening of the survival time and increased liver uptake. Asymmetrical appearance of intravascular platelet deposition may be due to anatomical alteration or variability.

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9. RESEARCH AND FUTURE PERSPECTIVES

Cellular labelling today is focused on stem cells and dendritic cells. Their clinical application has gained extensive interest, yet the fate of the cells and their kinetics is mostly unknown. Monitoring the fate of the cells and the peptide not only helps in the better understanding of the kinetics, but may also contribute to improving clinical application.

9.1. RADIOLABELLED STEM CELLS

Stem cells are clonogenic pluripotent progenitor cells that have a self-renewal capacity and can differentiate into specialized cells. They can be used for transplantation of bone marrow cells, treatment of neurodegenerative disorders, reduced myocardial perfusion, leukaemia, bone tissue repair and gene therapy [9.1, 9.2] (see Table 9.1). The use of stem cells follows from a great need to find a source of new healthy tissue to treat or to replace diseased or damaged human organs. Recently, genetic transfection of autologous stem cells has become a new tool in the therapy of genetic disorders [9.20]. Owing to the risks that xenogeneic and allogeneic donor stem cells may bring (graft versus host disease rejection, finding suitable donors, transmission of diseases and ethical issues with embryonic stem cells) autologous stem cells taken from bone marrow and adipose tissue are preferable for research.

There are strict regulations for stem cell labelling and use in humans, as they are considered genetically modified therapeutic tools. Therefore, before starting the procedure, there needs to be a clear understanding of local regulations and recommendations.

Although radiolabelling cells can induce damage to DNA, mesenchymal stem cells possess a good antioxidant reactive oxygen species scavenging capacity and active double strand break repair to facilitate their radioresistance [9.21]. Several methods can be applied for the radiolabelling of stem cells [9.22–9.24]. Indium-111-oxine labelling leads to high yields of labelling (67–86%) of (1–20) $\times 10^6$ pig or canine mesenchymal stem cells or rat endothelial progenitor cells which remained viable (>74–95%) after labelling and allows monitoring of the labelled cells in pigs, dogs and rats up to 14 days after injection. Some studies report a significant efflux (61–82% after 48–96 h) of radioactivity from the cells [9.25, 9.26]. High radioactivity in lungs or liver may indicate substantial damage to radiolabelled stem cells [9.27, 9.28]. Some also report ^{111}In induced cell damages (32–50% viability) to labelled cells [9.21, 9.28, 9.29], although not confirmed by others [9.30, 9.31]. In conclusion, radiolabelling stem cells with

TABLE 9.1. CLINICAL AND EXPERIMENTAL APPLICATIONS OF HAEMATOPOIETIC STEM CELLS

Application	Reference
Stem cell therapy for cancer	[9.3]
Rheumatic disorders and arthritis	[9.4]
Parkinson's disease	[9.5]
Myelin diseases	[9.6, 9.7]
Muscular dystrophies	[9.8]
Neurological disorders	[9.9]
Cardiac disorders	[9.10–9.12]
Vascular tissue regeneration	[9.13]
Severe combined immunodeficiency diseases	[9.14]
Treatment of diabetes and islets of Langerhans transplantation	[9.15–9.17]
Immunomodulatory regeneration and potentiation	[9.18]
Gene therapy with genetically modified stem cells	[9.19]

^{111}In-oxine is feasible, with high labelling yield but limited stability. In most studies, the integrity of the cells is affected, with substantially reduced viability and proliferation and limited homing and engraftment after transfusion of the radiolabelled cells. However, these parameters should be checked in each laboratory prior to human studies.

Technetium-99m-HMPAO has also been used for labelling mesenchymal stem cells from the bone marrow of rats and has remained highly viable (99%). Labelled cells have also been injected intravenously or infused into the left ventricular cavity of the heart in rats with an infarcted myocardium. High accumulation of radioactivity in the lungs (53% of the injected activity) with intraveneously administered labelled cells was observed, but this was much lower (2%) with labelled cell directly injected into the left ventricle [9.32]. Unfortunately, less than 1% of the transplanted cells resided in the infarcted heart 4 h after infusion. Severe damage to DNA with ^{99m}Tc-HMPAO labelling of stem cells has not been reported. The small number of cells (usually fewer than 10^7) result in poor labelling yields, and significant release of the radiolabel from the cells may occur, as it has been described for human white blood cells.

For preclinical research with radiolabelled stem cells, a ^{99m}Tc-tropolone labelling method has been developed [9.33]. Compared with ^{99m}Tc-HMPAO labelling of cells, this method is easy, fast and reproducible, without affecting

the viability and biological performance of the stem cells. Validation was performed in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice and showed that human cord blood $CD34^+$ stem cells, after labelling with ^{99m}Tc-tropolone, exhibit bone marrow homing capabilities [9.34]. After stimulation with a thrombopoietin growth factor, the radiolabelled stem cells accumulate in significantly higher numbers in the bone marrow compared with untreated cells.

There is interest in extending stem cell trafficking to PET. Although cells can be labelled with ^{18}F, its half-life and stability has not been optimized, and there is relative high uptake of radioactivity in the liver [9.35, 9.36]. However, much progress has been made with FDG labelled haematopoietic stem cell engraftment in the heart of patients with myocardial infarction, although the short physical half-life (110 min) is a disadvantage for the long term monitoring of stem cell trafficking [9.37]. In addition, the 30–40% washout at 2 h postlabelling is a limitation in the use of this tracer [9.38]. Other radiometals for labelling stem cells, such as ^{64}Cu (physical half-live 12.7 h), are being investigated [9.39]. Quality controls also need to be standardized [9.33].

In summary, various cells and various labelling methods have been investigated. Oxine and tropolone labelled with ^{111}In, ^{99m}Tc-HMPAO and FDG have been attempted for radiolabelling [9.40–9.44]. Age is negatively correlated to the number of cells harvested. Data on labelling vary widely (25–75%). Optimal conditions (10–30 min, 22°C or 37°C) are under debate. The results for tropolone and oxine are identical. The elution amounts to about 1%/h (for ^{111}In) versus 5%/h (for ^{99m}Tc).

These findings show that stem cells can be labelled without negatively affecting viability and the capacity to differentiate, but the route of application is the key issue. Although viability apparently is reasonably preserved, a final recommendation as to the optimal radiolabelling conditions cannot be given at present. There is great space for future studies. Insufficient information is available on which cell type, homing and maturity of the cell should be used.

9.2. RADIOLABELLED DENDRITIC CELLS

Dendritic cells are the most potent antigen presenting cells that play a key role in initiating and activating immune response. Several methods to load dendritic cells in vitro with tumour associated antigen peptides have been reported [9.45]. The radiolabelling data so far from the small number of experiments available do not allow for the final judgment on the optimal conditions. After injection of mesenchymal cells, the biology of dendritic cells is significantly modified and homing reduced.

9.3. OTHER RADIOLABELLED AUTOLOGOUS CELLS

Other cells have been isolated (e.g. eosinophils, basophils and monocytes) but have not yet found a way to clinical application.

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Appendix I

DOSIMETRY OF PROCEDURES

Appendix I reports the available dosimetric evaluations for white blood cells, red blood cells and platelets in adults and children.

I.1 DOSIMETRY OF RADIOLABELLED WHITE BLOOD CELLS

Based on available data, whole body and organ dosimetry for 225–250 MBq of FDG labelled leukocytes are comparable to those obtained with conventional activities of ^{111}In labelled leukocytes (see Tables I.1 and I.2).

TABLE I.1. RADIATION DOSIMETRY IN ADULTS

Radiopharmaceutical	Administered activity (MBq)	Organ receiving the largest radiation dose (mSv/MBq)	Effective dose equivalent (mSv/MBq)
In-111 leukocytes	10–18.5	Spleen 5.5	0.59
Tc-99m-sulphur colloid	300–370	Spleen 0.077	0.014
Tc-99m-HMPAO	185–370	Spleen 0.15	0.017

Source: See pp. 180, 232 and 256 of Ref. [I.1].

TABLE I.2. RADIATION DOSIMETRY IN CHILDREN (5 YEARS OLD)

Radiopharmaceutical	Administered activity (MBq)	Organ receiving the largest radiation dose (mSv/MBq)	Effective dose equivalent (mSv/MBq)
In-111 leukocytes	0.15–0.25	Spleen 17	1.8
Tc-99m-sulphur colloid	4.0–5.3	Spleen 0.25	0.014
Tc-99m-HMPAO	3.7–7.4	Spleen 0.48	0.054

Source: See pp. 180, 232 and 256 of Ref. [I.1].

I.2 DOSIMETRY OF RADIOLABELLED RED BLOOD CELLS

The dosimetry of labelled red blood cells varies according to the blood volume in total and individual organ (see Tables I.3 and I.4).

TABLE I.3. RADIATION ABSORBED DOSE ESTIMATES IN ORGANS FOR ^{99M}TC LABELLED RED BLOOD CELLS

Organ	From cell kinetics (mSv/MBq)	From organ blood volume (mSv/MBq)
Total body	0.016	0.005
Spleen	0.018	0.01–0.017
Blood	0.052	
Red marrow	0.022	
Liver		0.01–0.026

Source: See Ref. [I.2].

TABLE I.4. RADIATION DOSIMETRY IN ADULTS

Radiopharmaceutical	Administered activity (MBq)	Organ receiving the largest radiation dose (mSv/MBq)	Effective dose equivalent (mSv/MBq)
Tc-99m labelled red blood cells	750–1100	Heart 0.023	0.0085

Source: See p. 253 of Ref. [I.1].

I.3 DOSIMETRY OF RADIOLABELLED PLATELETS

The dosimetry of ^{111}In labelled platelets is calculated assuming an initial liver uptake of 8.5% and a splenic uptake of 23.5% (see Tables I.5 and I.6).

TABLE I.5. RADIATION ABSORBED DOSE ESTIMATES IN ORGANS FOR ^{111}IN LABELLED PLATELETS

Organ	Adult (mSv/MBq)	10 yrs old (mSv/MBq)	5 yrs old (mSv/MBq)	1 yr old (mSv/MBq)
Total body	0.52	1	1.6	2.7
Spleen	5.2	11	17	29
Ovaries	0.4	0.27	0.42	0.76
Testes	0.09	0.17	0.27	0.49
Red marrow	0.2	0.34	0.55	1.1
Liver	0.56	1.1	1.5	2.6

Note: The data were not upgraded in the last ICRP publication on this subject [I.3].
Source: See Ref. [I.4].

TABLE I.6. RADIATION DOSIMETRY IN ADULTS AND CHILDREN (5 YEARS OLD)

Radiopharmaceutical	Administered activity (MBq)	Organ receiving the largest dose (mSv/MBq)	Effective dose equivalent (mSv/MBq)
In-111 platelets			
Adults	10–18.5	Spleen 9.05	5.2
Children (5 yrs)	0.15–0.25	Spleen 0.444	17.0

Note: Radiation dose estimates to adults and children from various radiopharmaceuticals.
Source: Radiation Internal Dose Information Center, Oak Ridge Institute for Science and Education. Revision date 30 April 1996.

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Appendix II

A METHOD FOR QUALITY CONTROL OF ^{99M}TC-HMPAO

II.1. EQUIPMENT

— Centrifuge with variable angle rotor;
— Dose calibrator;
— Vortex.

II.2. MATERIAL

— Radioactivity protection shields;
— Sterile 10 mL vials;
— Gloves;
— Plastic pipettes.

II.3. REAGENTS

— NaCl 0.9% (saline);
— Chloroform.

II. 4. PROCEDURE

— In a 10 mL vial, prepare 3 mL of 0.9% NaCl (saline) and 3 mL of chloroform.
— Add 0.5 mL of freshly prepared ^{99m}Tc-HMPAO and mix using a vortex.
— With the vial in the upright position, wait until the two liquid phases have completely set.
— Count total radioactivity in the vial.
— Remove 1 mL of supernatant using a pipette and dispense it in a second vial.
— Count radioactivity in the second vial.

The percentage of lipophilic and hydrophilic HMPAO can be obtained using the following formula:

$$\text{labelling efficiency}\,(\%) = \frac{(\text{radioactivity in supernatant} \times 3)}{\text{total radioactivity}} \times 100 \qquad \text{(II.1)}$$

Appendix III

IN VITRO QUALITY CONTROL OF RADIOLABELLED WHITE BLOOD CELLS

Appendix III describes easy protocols for in vitro quality controls of radiolabelled cells (mainly white blood cells) that should be performed to validate or to revalidate the preparation of radiolabelled leukocytes.

All tests should always be performed following the specification detailed for the validation of the protocol, as defined in internal SOPs. Moreover, each test described in the pharmacopoeia retains its value only if performed following the indications described in the pharmacopoeia.

III.1. LABELLING EFFICIENCY

A useful parameter for the validation phase of labelled cells is the evaluation of the activity bound to cells relative to the total activity used for the labelling. It should be performed routinely before injecting the labelled cells into the patient. It is also referred to as labelling recovery.

III.1.1. Material and instrumentation required

— Dose calibrator.

III.1.2. Methodology

After the labelling is complete, set the instrument on the desired isotope and count the activity present in the syringe before the injection into the patient (bound). Then, count the supernatant of the last centrifugation (free):

$$\text{labelling efficiency}\,(\%) = \frac{\text{bound}}{\text{bound} + \text{free}} \times 100 \qquad \text{(III.1)}$$

III.1.3. Normal values

Each centre should calculate a mean value and a range of acceptability of this parameter, depending on the radiopharmaceutical and on the applied methodology.

It may be helpful to collect all data in a database to verify the trend of recovery over time.

It is important to know that the amount of radiopharmaceutical that enters in the cells, by a saturable passive transport, is influenced by many factors that depend on the patient (inflammation and therapy) and the technique (pH, viscosity, temperature and isotope purity).

Overall, an acceptable range of recovery would be in the ranges of 40–80% for ^{99m}Tc-HMPAO labelled white blood cells and 50–80% for ^{111}In-oxine labelled white blood cells. If recovery is <40% or <50%, further quality controls should be performed, such as microscopic inspection and trypan blue exclusion test for cell viability.

III.1.4. Impurity inspection

This type of quality control is to be routinely performed and comprises of the visual inspection of the solution by light contrast. It is useful to prove the absence of clumps, clots, cell aggregates or fibrin contamination.

At the end of the procedure, and before collecting the labelled cells in the syringe to administer to patients, the inspection should be performed carefully by gently rotating the vial. In the case of aggregates, they should be dissolved by gently shaking or pipetting the sample. If clumps cannot be dissolved, the preparation should not be injected.

III.2. RADIOPHARMACEUTICAL PURITY OF THE FINAL PRODUCT

This is a quality control of the final product to be performed occasionally for validation and revalidation of the whole procedure. It consists of the calculation of the activity bound to cells relative to the total activity administered. It can be calculated together with labelling recovery fraction.

III.2.1. Material and instrumentation required

— Centrifuge;
— Gamma counter/well counter;
— 0.9% NaCl (saline) solution;
— 2 vials;
— 10 μL pipette;
— 1 mL pipette;
— 0.5 mL pipette;
— Pipette tips.

III.2.2. Methodology

(1) Add 10 μL of the cell suspension, before the injection in the patient, in a vial (marked with a B, for bound) that contains 1 mL of 0.9% NaCl (saline) solution.
(2) Centrifuge at 2470g for 5 min.
(3) Collect 0.5 mL of the supernatant and transfer in another vial (marked with an F, for free).
(4) Count both vials in a gamma counter.

$$\text{radiopharmaceutical purity}\,(\%) = \frac{B - F}{B + (F \times 2)} \times 100 \qquad \text{(III.2)}$$

III.2.3. Normal values

The acceptability limit is ≥95%. If lower values are obtained further quality controls should be performed and the procedure needs further validation.

III.3. OPTICAL MICROSCOPE OBSERVATION OF LABELLED CELLS

This type of quality control is to be performed occasionally or for method validation and revalidation. It consists of verifying the type and morphology of labelled cells with an optical microscope. It enables the examination for the absence of microscopic cell aggregation, agglutination, clumps, cell damage or other cell activation markers. It is better to perform the standard staining with May Grünwald–Giemsa to visualize the different cell subsets.

III.3.1. Material and instrumentation required

— Optical microscope;
— Pre-stained slides for May Grünwald–Giemsa;
— 10 μL pipette.

III.3.2. Methodology for the use of pre-stained slides

(1) Apply one drop (10 μL) of the cell suspension (ready to be administered to the patient) in the centre of the glass slide.
(2) Cover with a cover slip and let the drop expand.
(3) Wait for 5 min.

(4) Observe the slide under the microscope following these criteria: examine at least three fields at 10x, 25x and 40x magnification.
(5) Evaluate the cell membrane, nuclei conservation and the presence of different cell types or aggregates.

III.3.3. Normal values

Only preparations without cellular microaggregates, agglutinations and with well preserved cell morphology can be accepted. If aggregates or damaged cells are detected, further quality controls should be performed, and the procedure needs further validation.

III.4. VITALITY TEST (TRYPAN BLUE TEST)

This type of quality control is to be performed occasionally and for method validation and revalidation. The test is performed with the trypan blue stain that enters inside dead cells and binds to DNA, giving these cells a blue stain. The observation under the optical microscope of several fields of the slide allows the detection of the presence of damaged or dead cells. It is one of the most important parameters to assess the stability of the preparation over time.

III.4.1. Material and instrumentation required

— Optical microscope;
— Cover slip;
— Glass slide;
— Bürker chamber;
— Trypan blue stain 0.4% (it is easier to use the available commercial solutions which are ready to use);
— Pipette.

III.4.2. Methodology

(1) Add 10–25 μL of the cell suspension ready to be administered to the patient in a small vial. If a Bürker chamber is being used, it is necessary to dilute the sample up to 1:100 with a 0.9% NaCl (saline) solution.
(2) Add 10–25 μL of the trypan blue stain 0.4%. Mix very well.
(3) Incubate for 5 min at room temperature.

For the visualization of the cells different methodologies can be used:

— The Bürker chamber allows the precise counting of cells and the calculation of the percentage of living cells relative to dead cells. It is an accurate method but requires a trained person for cell counting.
— If there is the need to perform the test routinely, it is preferable to use a glass slide that allows a rapid visualization. The Bürker chamber can be used if there are some doubts.

III.4.3. Normal values

A preparation with a percentage of dead cells (blue stained cells) of >4% should not be released for injection into the patient and, consequently, new tests for validation of the method should be undertaken.

III.5. CELL RECOVERY TEST

This type of quality control test is to be performed occasionally and for validation of the method. The test consists of counting the cells that are obtained during the different phases of the cell separation and labelling process. Since the number and the physiology of the cells can vary in each preparation, it is necessary to have an internal standard in order to obtain reproducible results.

III.5.1. Material and instrumentation required

— Disposable vials;
— Coulter counter;
— Cytofluorimeter.

III.5.2. Methodology

(1) During the blood sampling, collect some blood in a small vial to perform a haemachrome.
(2) At the end of the sedimentation, transfer some drops of cell suspension in a second vial.
(3) At the end of the first centrifugation, transfer some drops of cell suspension in a third vial.
(4) At the end of the labelling procedure, transfer some drops of cell suspension in a fourth vial.

For cell counting, a Coulter counter or a cytofluorimeter can be used. The latter is preferable, since it also provides information on the morphology of cells.

— Count the cells in each vial.

III.5.3. Acceptability limits

Table III.1 provides examples of acceptable limits for performing acceptable cell labelling.

TABLE III.1. ACCEPTABLE LIMITS FOR BLOOD CELL TYPES DURING THE PREPARATION OF LABELLED WHITE BLOOD CELLS FROM BLOOD SAMPLE TO INJECTION OF LABELLED CELLS (MEAN VALUE AND RANGE)

	Basal (in peripheral blood)	In vitro (after RBC[a] sedimentation) in 20 ml serum	Labelled cells ready to be injected in 5 ml	Other cell type to WBC[b] ratio
White blood cells (10^3/mm^3)	9.4 (4–15)	55.4 (30–150)	137 (70–350)	
Granulocytes (%)	84.3 (45–95)	89.2 (60–100)	91.9 (80–100)	
Lymphocytes (%)	12.8 (10–45)	8.3 (5–20)	6.4 (2–15)	
Red blood cells (10^6/mm^3)	4.4 (4–6)	0.13 (0.05–0.5)	0.2 (0.05–0.5)	<3
Platelets (10^3/mm^3)	246 (140–400)	115 (50–200)	120 (50–200)	<1

Source: Adapted from Ref. [III.1].

[a] Red blood cells.
[b] White blood cells.

III.6. ELUTION OF THE TRACER FROM THE CELL

This type of quality control is to be performed occasionally and for validation of the method. The lipophilic complexes can passively enter the cell. The isotope binds to the cellular components and, over time, leaves the cell. The test allows the verification of the percentage of tracer released from cells over time.

III.6.1. Material and instrumentation required

— Vials;
— Pipette;
— Tips;
— Gamma counter/well counter;
— Water bath.

III.6.2. Methodology

(1) Calculate the activity bound to the cells and in supernatant at different time points up to 24 h.
(2) Incubate labelled cells in water bath at 37°C in approximately 10 mL of culture medium and take 1 mL aliquots at several time points (15 min, 30 min, 45 min, 1 h, 2 h and 3 h). Each aliquot needs to be centrifuged and cells and supernatant counted separately.

III.6.3. Acceptability limits

At the end of the labelling, an injectable product needs to have at least 95% of the activity bound to the cells. Over time, this percentage decreases, and each centre should set its own acceptability limits in order to know the maximum period of time that can pass before administering to patients.

III.7. STERILITY TESTS

For post-release sterility testing, tests should be performed in accordance with the method described in the most recent European Pharmacopoeia. This test is preferably performed by a microbiologist, and may vary from centre to centre according to local needs and experience. The sterility test needs to be performed in triplicate for the validation of the procedure and in case of any modification to the procedure, including new personnel and new reagents. The recent availability of media fills allows validation and revalidation of the labelling procedure with sterility control of each single step of the labelling procedure. If sterility tests are not passed, the process needs to be revalidated.

Since the assay is completed after several days, it is not possible to use this test before administering labelled cells into patients, and it is possible to release the radiopharmaceutical without performing the test every time. The assay needs to be performed under aseptic conditions using a laminar flow hood (class A) placed in a sterile chamber (class B) or an isolator. The precautions that are used to

keep the aseptic conditions should not interfere with the microorganisms detected by the test. The media should satisfy the fertility assay. It is necessary to perform a validation assay, for the radiopharmaceutical of interest, of the methodology to show that the product has no antimicrobial activity in the conditions of the assay.

Nuclear medicine units usually ask the hospital microbiology to perform the test. The hospital microbiology should proceed to incubate the samples and analyse and understand the results following the pharmacopoeia specifications. In general, the samples should be incubated in thermostats for 7 days at 22.5 ± 2.5°C and for 7 days at 33.5 ± 2.5°C.

III.7.1. Material and instrumentation required

— Laminar flow hood/isolator;
— Culture broth/media ready to use or self-made following the pharmacopoeia specifications;
— Incubators.

III.7.2. Methodology

III.7.2.1. Media preparation

If ready to use media are used, it is necessary to verify whether they are appropriate to culture aerobic bacteria and mycetes. It can be useful to keep production certificates of the media. If media prepared in a laboratory are used, it is necessary to perform the fertility assay following the pharmacopoeia specifications.

III.7.2.2. Inoculation of the sample

(1) Inoculate the sample in two flasks with culture media.
(2) Add to one flask, marked as positive control, a small number of living microorganisms (10–100 CFU, colony forming unit) following table 2.6.1.-1 of Ref. [III.2].

The assay can be performed routinely inoculating the sample in flask with the same media and the same operative conditions used in the validation phase.

III.7.3. Acceptability limits

If a rapid and clearly visible bacterial growth is observed in the flask that contains the sample and the strain of living microorganisms (positive control),

the tested product does not possess any antimicrobial activity in the condition of the assay. The assay can be performed routinely without modifications.

If a rapid and clearly visible bacterial growth is not observed in the flask that contains the sample and the strain of living microorganisms (positive control), the tested product possess antimicrobial activity in the condition of the assay. The assay cannot be performed routinely, but the antimicrobial activity needs to be eliminated and the validation assay needs to be repeated. The flask that contains only the sample needs to be sterile. If not, the microorganisms need to be identified.

III.8. DETERMINATION OF BACTERIAL ENDOTOXINS

A test for contamination of solutions and reagents with pyrogens (Limulus test and Limulus amebocyte lysate (LAL) test) can also be used in addition to sterility tests. The method is described in detail in section 2.6 of Ref. [III.2].

REFERENCE TO APPENDIX III

[III.1] DE VRIES, E.F.J., et al., Guidelines for the labelling of leucocytes with ^{99m}Tc-HMPAO, Eur. J. Nucl. Med. Mol. Imaging **37** (2010) 842–848.

[III.2] EUROPEAN DIRECTORATE FOR THE QUALITY OF MEDICINES AND HEALTHCARE, European Pharmacopoeia, 8th edn, Council of Europe, Strasbourg (2013).

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The clinical use of radiolabelled autologous blood cells is considered of value for medical research and the diagnosis of disease. However, the labelling procedure is time consuming, relatively expensive and exposes the operator and patient to several risks. Standardizing radiolabelling methods for autologous blood cells can help to provide international standards for quality assurance and control and to summarize indications, norms and ethical considerations in the clinical use of radiolabelled cells. This publication provides a framework for the standardization of the methods using radiolabelled autologous blood cells, including dendritic cell labelling and stem cell labelling for their particular importance in medical research and for their great potential for clinical applications.

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