Measuring the whole bone marrow asset in humans by a computational approach to integrated PET/CT imaging.

Gianmario Sambuceti[1,2,3], Massimo Brignone[1,4], Cecilia Marini[2], Michela Massollo[1], Francesco Fiz[1], Silvia Morbelli[1], Ambra Buschiazzo[1], Cristina Campi[5], Roberta Piva[1], Anna Maria Massone[7], Michele Piana[4,7] and Francesco Frassoni[6,3]

[1]Nuclear Medicine, Dept. of Internal Medicine, University of Genoa, Genova, Italy; [2]CNR Institute of Bioimages and Molecular Physiology, Genova, Italy; [3]Advanced Biotechnology Center, Genova, Italy; [4]Department of Mathematics, University of Genoa, Italy; [5]Department of Computer Science, University of Helsinki, Finland; [6]Stem Cell Center, San Martino Hospital, Genova, Italy; [7]CNR-SPIN. Genova. Italy

Running Head: PET/CT measurement of bone marrow volume

Address for Correspondence
Gianmario Sambuceti
Nuclear Medicine. Department of Internal Medicine
University of Genoa
Viale Benedetto XV. 6
16132 Genova-Italy
Phone: +39 010 555 2027 Fax: +39 010 555 6911
Sambuceti@unige.it

Abstract

Purpose. Despite their relevance in clinical medicine, extension and activity of bone marrow (BM) cannot be directly evaluated in vivo. This study proposes a new method to estimate these variables by combining structural and functional maps provided by X ray computed tomography (CT) and positron emission tomography (PET).

Methods. BM extension and glucose uptake were estimated in 102 patients submitted to whole body PET/CT because of a history of non-metastatic melanoma. Image analysis assumed that BM is surrounded by compact bone. An iterative optimization scheme was applied to each CT slice to recognize the external bone border. To identify the compact bone, the algorithm measured the average Hounsfield coefficient within a 2-pixel ring located just inside thebone contour. All intraosseous pixels having attenuation coefficient lower than this cutoff were flagged 1, while the remaining ones were set at 0. Binary masks created from all CT slices were thus applied to PET data to extract the metabolic activity of the intraosseous volume (IBV).

Results. Whole body IBV was 1632±587 cm^3 and was higher in males than in females (2004±498 cm^3 vs 1203±354 cm^3. $p<0.001$). Overall, IBV was strictly correlated with ideal body weight ($r=0.81$. $p=0.001$) and only loosely with measured body weight ($r=0.43$. $p=0.01$). Average FDG standard uptake value (SUV) in thoracic and lumbar vertebrae was 2.01±0.36. Accordingly, intraosseous voxels with SUV≥1.11 (mean spine SUV – 2.5 SDs) were considered as active, "red" BM while those with SUV <1.11 as "yellow" BM. Red BM volume was 541±195 ml with a higher prevalence in axial than in appendicular skeleton (87±8% vs 10±8%. $p<0.001$). Red BM volume was higher in males than in females (7.8±2.2 vs 6.7±2.1 ml/Kg body weight. $p<0.05$), yet in the latter it occupied a greater fraction of IBV (32±7% vs 36±10%. $p<0.05$). Age modestly predicted red BM SUV, while it robustly and inversely correlated with red BM volume.

Conclusions. Our computational analysis of PET/CT images provides a normalcy range of BM extension and metabolism. This information might represent a new window to explore BM pathophysiology and its response to chemotherapy.

Keywords: PET/CT; bone marrow imaging; image processing.

Introduction

Bone marrow (BM) is a complex and dynamic organ, affected by almost every hematologic disease and involved in a large number of oncologic, degenerative and inflammatory disorders. Its study has been markedly improved in the last decades, yet biopsy remains the gold standard procedure for its evaluation [1] and to reach a specific diagnosis. However, due to the "regional" nature of sampling, its value intrinsically postulates a homogeneous distribution of BM biology. Nevertheless, this assumption is challenged by the frequent observation of discrepancies between BM histology and patient's laboratory and clinical findings. The capability of sampled tissue to represent the whole hematopoietic system might be thus suboptimal, particularly in those patients in which BM involvement is largely heterogeneous. Under these conditions, diagnostic accuracy would be largely improved by methods able to complement the evaluation of molecular biology and phenotype of cells populating marrow biopsy or aspiration.

Actually, what we intrinsically miss in each given patient is some quantitative information about BM extension as well as a complete definition of its distribution throughout the skeleton. In the past decades, this evaluation has been attempted with nuclear medicine methods [2], using tracers targeting the reticuloendothelial system [3] as well as erythropoietic [4] or granulopoietic cells [5]. These techniques offered the advantage to directly interrogate specific BM functions, however, they can not estimate BM volume because of intrinsic limitations in both tracer kinetics and physics of single photon detection. Theoretically, the capability of MRI to estimate water and fat content with an excellent spatial resolution might provide an accurate evaluation of red and yellow BM throughout the intraosseous space [6-9]. However, the complexity of acquisition modality and the influence of signal distortion prevented the development of standardized clinical protocols able to extend this evaluation to the whole body limiting the use of MRI to characterize specific bone districts.

A different window on BM function has been recently introduced by the widespread distribution of positron emission tomography and 18F-fluoro-deoxyglucose (FDG-PET). In particular, the introduction of hybrid PET-CT systems and the consequent coregistration of functional and anatomical maps markedly improved our capability to characterize BM metabolism [2, 10-12]. This method has been successfully used to document BM infiltration by cancer [13] as well as to monitor the hematopoietic response to chemo or radiotherapy [14]. However, due to the absence of method to evaluate BM extension most of these studies limited their analysis to the metabolic activity of specific bone segments taken as samples of the whole tissue. As a consequence, no data are available so far about the human BM global asset under physiological or pathological conditions.

In the present study, we describe and propose a novel computer algorithm able to discriminate intraosseous space on CT images and to extract BM metabolic activity from co-registered PET data. This analysis was applied to a large population of normal subjects to obtain a "normalcy" data base of BM extension and distribution in the whole skeleton as well as in specific bone segments.

Materials and Methods

Study population

The study group included 102 consecutive subjects studied by whole body FDG-PET/CT imaging from January to December 2009 for the assessment of disease activity of non-metastatic melanoma (Table 1). Selection criteria were: 1) histologic diagnosis of melanoma completely removed, subsequent negativity of sentinel lymph node histology and absence of whatever evidence of disease relapse at least two years after surgery; 2) normal serum creatinine levels; 3) no history of diabetes and serum glucose level ≤1g/L at the time of injection and 4) documented radiochemical purity of injected tracer >98%. Exclusion criteria included previous chemotherapy, radiation, or immunosuppression therapy, confirmed metastases at any site, active infection or inflammation and any evidence or history of hematologic disease. All available results from other imaging investigations (MRI, CT, ultrasonography and conventional radiography), laboratory data, biopsies, and the clinical course were taken into consideration to rule out any evidence of further disease.

PET/CT acquisition

After a minimum of 12 hours fasting. serum glucose level was measured before the intravenous injection of 4.8-5.2 MBq of ^{18}F-fluorodeoxyglucose (FDG) per kilogram of body weight. At the time of the study, weight and height of all patients were measured; ideal body weight was estimated according to the conventional formulation [15]. FDG-PET imaging started 60 to 75 minutes after tracer administration and was performed using an integrated PET/CT scanner (Hirez; Siemens Medical Solutions. Knoxville TN. USA). The entire body – from skull vertex to toes – was acquired in all patients.

In Vivo Image Analysis

PET raw data were reconstructed by means of ordered subset expectation maximization (OSEM) and attenuation correction was performed using the CT data. The entire CT dataset was coregistered with the 3D PET images using an integrated software interface (Syngo; Siemens Erlangen. Germany) to create anatomical images superimposed with FDG ones.

CT information was then processed in order to determine the global intraosseous volume potentially available for BM. The algorithm is based on two assumptions: 1) the intrabone volume (IBV) is surrounded by compact

bone and 2) Hounsfield value (i.e. the degree of tissue X-ray absorption) is highest in compact bone among all tissues. However. since attenuation coefficients differs in bones belonging to different districts. a mere application of a single cut-off value to allow automatic identification of the whole bone tissue in all CT slices is impossible. A more sophisticated computational procedure was then devised and introduced, that is based on three steps as schematized in Figure 1:

Step 1. In each CT axial image. an optimization algorithm based on a level set technique [16] is applied in order to automatically identify the external bone contour.

Step 2. A thresholding procedure partitions the obtained region into compact and trabecular bone according to the attenuation coefficient of each pixel.

Step 3. A binary mask is created, exported from the CT framework and pixel-wise multiplied against the PET co-registered data in order to extract BM metabolic activity.

More precisely, the process starts with the unique human intervention and asks the operator to draw a loose region around the skull vertex as a starting reference. Then, the functional representing the energy of a curve surrounding the bone profile is iteratively minimized thus determining a sequence of active contours that progressively adapt themselves onto the external bone border. As described by Chan et al [16], this algorithm assumes a deformable model that defines an active contour as the zero level set of a function ϕ: $\phi: \mathbb{R}^2 \to \mathbb{R}$. The energy functional, to be minimized, is given by

$$F(\phi) = \left(\int \delta(\phi) |\nabla \phi| dx \right)^2 + \int (I - m_1(\phi))^2 H(\phi) dx + \int \left(I - m_2(\phi)\right)^2 (1 - H(\phi)) dx$$

where the integrals are computed in the domain of the image, and adopted symbols are as follows: $H: \mathbb{R} \to \mathbb{R}$ is a regularized version of the Heaviside function [17], $\delta: \mathbb{R} \to \mathbb{R}$ is a regularization of the Dirac delta [18], m_1 and m_2 are ϕ dependent constants defined as

$$m_1(\phi) = \frac{\int I\, H(\phi) dx}{\int H(\phi) dx} \quad \text{and} \quad m_2(\phi) = \frac{\int I\, (1 - H(\phi)) dx}{\int 1 - H(\phi) dx}$$

Minimization of F can be thus obtained by treating the associated Euler-Lagrange equation [19] as a dynamic equation, i.e. by solving the evolution problem

$$\frac{\partial \phi}{\partial t} = \delta(\phi) \left[2\, div \left(\frac{\nabla \phi}{|\nabla \phi|} \right) \int \delta(\phi) |\nabla \phi| dx - \left(I - m_1(\phi)\right)^2 + (I - m_2(\phi))^2 \right]$$

A numerical solution to the previous differential equation can be obtained discretizing both in space and time and solving iteratively (in t) the discretized system until a stable solution is found. Once the algorithm reaches the convergence, and denoted by ϕ^* the solution, the region inside the active contour – and thus the bone plus intrabone area – will be identified by the set

$M = \{x \in \mathbb{R}^2 \mid \phi^*(x) \leq 0\}$.

The second – thresholding – step, starts from the external profile of each area M_k identified by the previous step. It samples a 2 pixel thick layer and computes its average Hounsfield value μ_k. The region of M potentially at disposal to BM is thus computed as:

$$P = \bigcup_k \{x \in M_k \mid I(x) \leq \mu_k\}$$

The region identified by the set P is used to create the projection function $\mathcal{P}: \mathbb{R}^2 \rightarrow \{0.1\}$ such that

$$\mathcal{P}(x) = \begin{cases} 1 & if\ x \in P \\ 0 & elsewhere \end{cases}$$

In the third step, this function is thus point by point multiplied against the PET co-registered map I^{PT} in order to extract and represent the BM metabolic activity without the confounding effect of tracer uptake in the rest of the body: the BM representation is than given by the function $I^{PT}\mathcal{P}$.

This procedure is automatically replicated to all slices. In particular, the optimized active contour obtained at the end of the processing of the first slice is utilized as initialization contour for the successive slice. The final 3D result is thus displayed to the operator for the removal of non bone calcified regions as well as for the check of the appropriate recongnition of the spinal canal as extraosseous space.

The output of the software is therefore the quantitative assessment and the 3D representation of three different volumes: 1) the whole skeleton; 2) the compact bone tissue and 3) the space potentially available for BM. The application of these procedures to all slices of acquisition permits to evaluate the whole skeleton and each slice to exclude non-bone calcified regions or possible inclusion of spinal canal.

The algorithm also allows the analysis of specific skeletal districts in order to facilitate the comparison with previous experiences on BM distribution and metabolic activity. To this purpose. the operator is asked to select a 3D volume of interest (VOI) on CT images and the three volumes are automatically detected to extract the corresponding PET metabolic information in that specific VOI. The skull is excluded from this analysis as well as from the whole body volume extraction, since the spillover of brain radioactivity prevents an accurate measurement of FDG uptake in the BM of this region.

Statistical analysis

All data are reported as means ± SD. Unpaired or paired t-test was used. as appropriate. Linear regression analysis was performed using the least squares method. P values <0.05 were considered significant.

Results

Extension and metabolic activity of bone marrow throughout the human body

Mean IBV in the entire population was 1631±592 ml with a highly significant difference between males and females (2004±498 ml vs 1203±354 ml, respectively, $p<0.001$). The volume was loosely correlated with measured body weight ($r=0.43$, $p<0.01$), while it was strictly predicted by ideal body weight ($r=0.81$, $p<0.001$) in both genders (Figure 2). Overall, IBV normalized for measured body weight was 22±7 ml/Kg and again it was higher in men than in women (25±7 vs 19±6 ml/Kg body weight, respectively, $p<0.001$).

The distribution of intraosseous space in the different body segments was calculated as % of the total IBV and is shown in Table 1. In summary, space available in the appendicular skeleton was higher with respect to that of the axial one, with only minor differences between the two genders.

The average standardized uptake value (SUV) within the intraosseous space throughout the body was 0.96±0.17; there were no differences between males and females (0.93±0.15 vs 0.99±0.19, respectively, p=ns). Obviously. this value represents the integrated average between all the components included in this volume that can be conventionally and classically defined as "red" active BM and "yellow" less active BM. To separate these two components, we first selected a skeletal district almost completely occupied by active red BM [8-10]: i.e. the soma of all thoracic and lumbar vertebrae. This region accounted for a volume of 243±67 ml and showed an average SUV of 2.01±0.36. Accordingly, red BM was defined on a statistical basis as all intraosseous voxels in all skeletal segments with FDG uptake greater than mean vertebral SUV – 2.5 SDs, i.e. with SUV $\geq$ 1.11. On the contrary, voxels with FDG uptake below this threshold were considered as yellow BM (Figure 3).

Extension and distribution of red BM throughout the skeleton

Overall, red BM volume had a lower extension than the yellow one (541±195 ml vs 1093±455 ml, respectively, $p<0.0001$) accounting for 34±9% of the total available intraosseous space. It was larger in men than in women (634±180 ml vs 435±156 ml, respectively, $p<0.001$) and correlated with both measured ($r=.57$. $p<0.01$) and ideal body weight ($r=0.6$. $p<0.01$). Red BM value normalized for measured body weight (7.2±2.2 ml/Kg body weight) was slightly higher in males than in females (7.8±2.2 vs 6.7±2.1 ml/Kg body weight. respectively, $p<0.05$), although the fraction of intraosseous space actually occupied by red BM was higher in the latter (32±7% vs 36±10%, respectively, $p<0.05$).

Red BM distribution within different regions was calculated as % of available volume in the corresponding skeletal segment: its prevalence and absolute extension are represented in Figure 4. As expected, BM type was largely dependent upon the analyzed skeletal compartment: in fact, red BM prevalence was markedly higher in the axial with respect to the appendicular skeleton (87±8% vs 10±8%, respectively, $p<0.001$) where it was

confined to the heads of humeri and femora (Figure 4 and 5). On the contrary, long bone shafts as well as tibiae were almost fully occupied by yellow BM (Figure 5). Gender did not significantly affect red BM distribution throughout the skeleton.

Metabolic activity of red BM

Obviously, red BM had a markedly higher mean SUV with respect to the yellow one (1.8±0.2 vs 0.5±0.06, respectively, $p<0.001$). Differently from morphological variables, metabolic pattern and thus SUV of red BM were similar in the two genders (1.84±0.17 vs 1.78±0.23, respectively, p=ns). Interestingly, metabolic activity of red BM in the different regions was distributed in a homogeneous fashion in all patients, with SUV in the axial skeleton consistently higher than in the appendicular segments (1.9±0.3 vs 1.2±.2. respectively, $p<0.01$). Overall, the whole intraosseous space accumulated 2.08±0.6% of injected dose. Red BM volume was three times lower whem compared to the yellow one. Nevertheless, due to its extremely high metabolic activity. it retained an almost double amount of available FDG (1.3±0.5% vs 0.7±0.3%, respectively, $p<0.001$).

Effect of age activity and extension of the bone marrow

Age had a significant effect on BM state. In fact as shown in figure 6, it only modestly predicted SUV in the red BM ($r=0.24$. $p<0.05$), while it robustly and inversely correlated with red BM volume expressed both as percentual occupancy of intraosseous space ($r=0.40$, $p<0.01$) and red BM mass normalized for body weight ($r=0.40$. $p<0.01$). As a consequence. the fraction of the dose eventually retained by active BM sites decreased over years ($r=0.42$. $p<0.01$).

Discussion

To the best of our knowledge, the present study represents the first attempt to simultaneously estimate extension and distribution of BM in living humans. This computational approach to co-registered PET-CT images simultaneously estimates IBV and BM metabolic pattern. Obtained data thus represents an unexplored window on extension, activity and distribution of the whole hematopoietic tissue bearing the potential to improve our understanding of BM pathphysiology in the course of malignancies or degenerative disorders.

Recognition and measurement of whole body intraosseous space by CT analysis

The estimation of intraosseous space throughout the whole body is based on the same assumption of commercially available softwares for 3D skeleton representation: i.e. the compact bone is the structure with the highest attenuation for X rays among human tissues [20]. However, different skeletal segments in different body regions display largely divergent attenuation coefficients. This variability does not permit to identify a

cutoff in Housfield values able to pursue this task throughout the whole body. This simplified criterion, in fact, would imply a human intervention that would hamper both analysis accuracy and measurement repeatability.

Once defined its external edge, the compact bone is defined as "the box" encompassing the space available for BM whose extension and location can be extracted applying the opposite criterion: i.e. identifying intraosseous voxels with the largest drop in attenuation coefficient. IBV measured with this method was strictly dependent upon ideal body weight indicating that the space available for BM colonization is mostly influenced by subject height and thus by genetically determined growth processes.

Although somewhat intuitive, this concept has never been evaluated in living humans. In fact, no method is so far avilable to measure whole body IBV whose current description [21-25] still relies on the measurements published by Mechanik in 1926 [21]. That study – extensively reviewed by Woodward [26], Ellis [27] and Cristy [28] – reports the BM mass in 13 subjects of different age and gender, died for non-hematologic diseases. The relative distribution of intraosseous space in the different skeletal districts reported in these papers agrees with our data. Conversely, our estimation of total BM volume was lower with respect to pathological findings: 25 ml (2.5%) vs 34-64 ml /Kg body weight (3.4-6.4%). Several factors might account for this difference. On one side Mechanik's approach implied the prolonged boiling of post-mortem harvested bones to estimate an index of BM mass while our method implies the metabolic imaging of living BM to measure its volume. Moreover, as a limitation of our method the skull was excluded from the analysis due to the contamination of radioactivity measurement originating from brain spillover. Finally, the divergent number and condition of the two study populations might have resulted in large differences in body mass.

Defining BM in living human: a new experimental picture explaining old postulates

The recognition of the two different marrow types started from the concept – documented both by pathology [21] and by imaging studies [2, 9-10] – that vertebral bodies are most largely occuplied by red BM. Actually, our approach documented that FDG uptake in this bone district is higher than in the rest of IBV and displays a Gaussian distribution. Accordingly, voxels with SUV <1.11 (average spine SUV minus 2.5 SDs) were considered to have a <0.05 probability to belong to red BM. Once extended to the whole skeleton, this criterion indicated that red BM occupies roughly one third of the whole IBV and presents a distribution that closely fits the current model derived from pathology [21-28].

Due to the greater body mass. red BM asset was larger in males than in females in whom, however, it occupied a greater fraction of available volume. This finding agrees with the increased need for hematopoiesis occuring in women during fertile age [7-8, 10, 29]. Even more interestingly, our analysis confirmed the current model of

red BM evolution during life. On one side, conversion from red to yellow tissue had a relatively homogeneous trend with appendicular bones getting progressively devoid from hematopoietic activity according to the so called Neumann law [30]. On the other hand, red BM showed a substantialy stable FDG uptake until elderly age, while its overall extension progressively decreased. It thus appears that the overall decline in BM SUV over years described in previous PET studies [10-11] could reflect a reduction in total red BM volume whose glucose consumption, as an indirect index of hematopoietic activity, remains relatively stable. From the physiological viewpoint, this observation suggests that BM cell production is modulated in two ways: by tuning the intensity of proliferating activity per unit mass or by modifying the total mass of productive BM. The effect of age in "normal subjects" discloses that the pathways active on these two mechanisms are at least partially independent. Accordingly, both pieces of information are needed to obtain an exhaustive description of hematopoietic function in health and disease.

Limitations

Several limitations of our approach to BM extension and to characterize BM biology have to be considered. The operator-independent measurement of BM volume is theoretically robust and reproduces the routine estimation of diameters and sizes in X ray CT. However, a significant number of osseous trabeculae display a thickness below the CT spatial resolution. The consequent partial volume averaging effect [31] might thus overestimate the "volume available for BM colonization". Overcoming this limitation and improving image details, would have implied to increase patient radiation burden raising obvious ethical concerns. However, this theoretical inaccuracy seems to play a minor role. In fact, our measurement of whole body IBV provided relatively lower values with respect to pathology [21], while on a regional basis, our data closely fit with lumbar vertebrae IBV measured by MRI in normal subjects [8-10]. Finally, increasing the anatomical definition would have scarcely influenced the accuracy of the relatively blurred functional assessment due to the limited structural detail of nuclear detection.

Similar considerations apply to the different temporal resolutions of CT and PET: the short acquisition time virtually eliminates motion artifacts from CT scan, while the longer PET acquisition time inevitably results in respiratory motion of both ribs and sternum [32]. Although this limitation might be at least partially solved by gating, the time needed to this purpose would hardly fit with the clinical routine in PET/CT labs.

Due to the dispersed nature of hematopoietic function, BM biology was evaluated by measuring the glucose consumption of tissues populating the extracted IBV. Obviously, glucose metabolism represents an indirect index of proliferating activity and cannot differentiate "per se" BM cell types. Moreover the numerical

representation (SUV) of FDG uptake is affected by a number of variables. From a technical point of view, patients with evidence of tracer extravasation were excluded, while quality control procedures were performed daily to correct for scanner efficiency as well as for its drift over time. From a pathophysiological point of view, FDG uptake depends upon tracer availability and thus is correlated directly with the time elapsed from injection to acquisition and inversely with glucose consumption in the rest of the body [33-34]. The former parameter can be accurately controlled and, for the purposes of the present analysis, seems of relatively minor importance given the relative stability of BM SUV up to hours after tracer injection [35]. On the contrary, differences in whole body metabolic pattern may occur even despite an accurate control of preliminary fasting and control of serum glucose level. Although this variability may actually limit the accuracy of proposed criteria to recognize the different BM types in each single patient, several considerations indicate that FDG uptake within the extracted IBV can perform this differentiation at least for population-based analyses. On one hand, our method reproduces the previously reported use of SUV to monitor hematopoietic function in the course of chemotherapy or in its subsequent recovery phase [14]. On the other hand, our procedure segmented vertebral bodies as samples of red BM [8-10] and provided SUVs quite similar to those reported by coregistration of PET and MRI at a micrometric level.

As final technical considerations, the selection of a fluorinated tracer implies an extreme care in documenting tracer radiochemical purity to avoid the cross talk of free fluorine accumulated in bone hydroxyapatite crystals [36]. However, the FDG/total activity ratio was always >98% as asked by clinical standards, thus limiting the interference of this contamination. Finally, the skull was not analyzed because of the spillover from the high brain radioactivity. Obviously, this limitation does not hamper the measurement of cranial intraosseous volume that could be used for PET imaging with tracers exploring different tissue functions [37]. However, the method was designed to allow the evaluation of BM volume using FDG, a tracer characterized by a large experience and by a widespread distribution. Accordingly, this approach can represent a reasonable compromise to allow the clinical exploitation of BM mass measurement in the routine practice of nuclear medicine centers.

Conclusions

The first application of our method aimed to build up a normalcy data base that can be used as a standard by different centers and provides a definition of marrow response to physiological variables active in the course of adult life. To this purpose, we selected the most normal population submitted to whole body PET/CT scan: subjects with history of first stage melanoma [38], having no evidence of local or remote disease. and

documented absence of relapse in at least two years. Although BM evolution in children was not assessed [39], our analysis confirms that active “red” BM does not occupy the whole available space in adulthood and displays a further progressive slow reduction until advanced age starting from the appendicular bones [8-11, 39-40].

The present approach represents a tool to provide clinical medicine with two largely unexplored data: the total IBV available for BM colonization and an index of hematopoietic activity distribution throughout the skeleton. Besides its intrinsic value, the former variable represents a valuable tool to expand the potential of PET/CT imaging for the investigation of BM especially for studies using tracers such as F-thymidine [37] able to interrogate specific functions of this tissue. The latter represents an integrated index potentially expanding the diagnostic capability of PET/CT to evaluate the hematopoietic function in response to chemotherapy applied for solid tumors. The sinergy of this integrated approach can thus provide a macroscopic picture of BM able to nicely complement the microscopic and molecular investigation of hematopoiesis and could possibly provide a new window for applying PET/CT imaging to the study of BM physiology in a number of disease conditions.

Acknowledgements

This work was supported by grants from AIRC. Compagnia di San Paolo Torino. FONDAZIONE CARIGE. Progetto LIMONTE). Ministero della Salute (Ricerca Finalizzata Ministeriale 2009). Regione Liguria. Ricerca Finalizzata anno 2009 and the Associazione Italiana Leucemie. Sezione Ligure.

References

1. Knowles S. Hoffbrand AV. Bone-marrow aspiration and trephine biopsy. *Br Med J.* 1980; 281:204-205.
2. Agool A. Glaudemans AW. Boersma HH. Dierckx RA. Vellenga E. Slart RH. Radionuclide imaging of bone marrow disorders. *Eur J Nucl Med Mol Imaging*. 2011; 38:166–178.
3. Desai AG. Thakur ML. Radiopharmaceuticals for spleen and bone marrow studies. *Semin Nucl Med* 1985;15:229–38.
4. Datz FL. Taylor A Jr. The clinical use of radionuclide bone marrow imaging. *Semin Nucl Med* 1985;15:239–59.
5. Wahren B. Gahrton G. Hammarstrom S. Nonspecific crossreacting antigen in normal and leukemic myeloid cells and serum of leukemic patients. *Cancer Res* 1980;40:2039–44.
6. Mitchell DG. Burk DL. Vinitski S. et al: The biophysical basis of tissue contrast in extracranial MR imaging. *Am J Roentgenol* 1987; 149:831-837.
7. Duda SH. Laniado M. Schick F. Strayle M. Claussen CD. Normal bone marrow in the sacrum of young adults: differences between the sexes seen on chemical-shift MR imaging. *Am J Roentgenol.* 1995; 164:935-940.
8. Kugel H. Jung C. Schulte O. Heindel W. Age- and sex-specific differences in the 1H-spectrum of vertebral bone marrow. *J Magn Reson Imaging.* 2001;13:263-268.
9. Basu S. Houseni M. Bural G. et al. Magnetic resonance imaging based bone marrow segmentation for quantitative calculation of pure red marrow metabolism using 2-Deoxy-2-[F-18]fluoro-D-glucose-Positron Emission Tomography: a novel application with significant implications for combined structure-function approach. *Mol Imaging Biol* 2007; 9:361-365.
10. Blebea JS. Houseni M. Torigian DA. et al. Structural and functional imaging of normal bone marrow and evaluation of its age-related changes. *Semin Nucl Med* 2007;37:185-194.
11. Fan C. Hernandez-Pampaloni M. Houseni M. et al. Age-related changes in the metabolic activity and distribution of the red marrow as demonstrated by 2-Deoxy-2-[F-18]fluoro-D-glucose-Positron Emission Tomography. *Mol Imaging Biol* 2007; 9:300-307.
12. Shreeve WW. Use of isotopes in the diagnosis of hematopoietic disorders. *Experimental Hematology* 2007; 35:173–179.
13. Kumar R. Alavi A. Fluorodeoxyglucose-PET in the management of breast cancer. *Radiol Clin N Am* 2004;42:1113–22
14. Jhanwar YS. Straus DJ The Role of PET in Lymphoma. *J Nucl Med* 2006; 47:1326–1334.

15. Robinson JD. Lupkiewicz SM. Palenik L. Lopez LM. Ariet M. Determination of ideal body weight for drug doseage calculation. *Am J Hosp Pharm* 1983; 40:1016-1019

16. Chan TF. Vese LA. Active contour without edges. *IEEE Transactions on Image Processing.* 2001; 10:266-277.

17. Abramowitz M and Stegun IA. *Handbook of mathematical functions*. National Bureau of Standards. Applied Mathematics Series. Washington DC. Tenth Printing. December 1972. p. 1020.

18. Dirac P. *Principles of quantum mechanics (4th ed.)*. Oxford at the Clarendon Press. 1958.

19. Courant R and Hilbert R. *Method of Mathematical Physics.* New York. Interscience. 1953:Vol. I.

20. Choplin RH. Farber JM. Buckwalter KA. Swan S. Three-dimensional volume rendering of the tendons of the ankle and foot. *Semin Musculoskelet Radiol.* 2004;8:175-183.

21. Mechanik N. Untersuchungen ueber das Gewicht des knochenmark des Menschen *Z Gesamte Anat* 1926; 79:58-99.

22. ICRP. Report of the Task Group on Reference Man. *ICRP Publication* 23. Pergamon Press. Oxford. UK. 1975.

23. Valentin J. Basic anatomical and physiological data for use in radiological protection: reference values: *ICRP Publication 89. Annals of the ICRP* 2002; 32:1-277

24. Bonner H. The blood and lymphoid organs. In: *Rubin's Pathology.* Lippincott. Phyladelphia 1988; p 1014

25. Atkinson HR. Bone marrow distribution as a factor in estimating radiation to the blood-forming organs: a survey of present knowledge. *J Coll. Radiol Aust* 1962; 6:149-154.

26. Woodard HQ. Holodny E. Summary of the Data of Mechanik on the Distribution of Human Bone Marrow. *Phys Med Biol* 1960; 5:57-59.

27. Ellis RE. The distribution of active bone marrow in the adult. *Phys Med Biol* 1961; 5:255-258.

28. Cristy M. Active bone marrow distribution as a function of age in humans. *Phys Med Biol* 1981; 26:389-400.

29. Custer RP. Ahlfeldt FE. Studies on the structure and function of bone marrow. II. Variations in cellularity in various bones with advancing years of life and their relative response to stimuli. *J Lab Clin Med* 1932; 17:960-962.

30. Tavassoli. M. Bone Marrow: The Seedbed of Blood. In Wintrobe MM: *Blood. pure and eloquent sh.5*. Mc.Graw-Hill Book Company. New York. 1980.

31. Yankelevitz DF, Henschke CI, Davis SD. Percutaneous CT biopsy of chest lesions: an in vitro analysis of the effect of partial volume averaging on needle positioning. Am J Roentgenol. 1993;161:273-278.

32. Aston JA. Cunningham VJ. Asselin MC. Hammers A. Evans AC. Gunn RN. Positron emission tomography partial volume correction: estimation and algorithms. *J Cereb Blood Flow Metab*. 2002; 22:1019-1034.

33. Thie JA. Clarification of a fractional uptake concept. *J Nucl Med*. 1995;36:711-712.

34. Thie JA. Understanding the Standardized Uptake Value. Its Methods. and Implications for Usage. *J Nucl Med.* 2004;45:1431-1434.

35. Chin BB. Green ED. Turkington TG. Hawk TC. Coleman RE. Increasing Uptake Time in FDG-PET: Standardized Uptake Values in Normal Tissues at 1 versus 3 h. *Mol Imaging Biol* 2009; 11:118-122.

36. Czernin J. Satyamurthy N. Schiepers C. Molecular mechanisms of bone 18F-NaF deposition. *J Nucl Med*. 2010; 51:1826-1829.

37. Agool A. Schot BW. Jager PL. Vellenga E. 18F-FLT PET in hematologic disorders: a novel technique to analyze the bonemarrow compartment. *J Nucl Med*. 2006; 47:1592–1598.

38. Balch CM. Gershenwald JE. Soong SJ. et al. Final Version of 2009 AJCC Melanoma Staging and Classification *J Clin Oncol*. 2009; 27:6199–6206.

39. Emery JL. Follett CF. Regression of bone marrow haemopoiesis from the terminal digits in the foetus and infant. *Br J Haematol* 1964; 10:485-489.

40. Vogler JB. Murphy WA. Bone marrow Imaging. *Radiology*. 1988; 168:679-693.