

Immunoscintigraphy of the Bone Marrow: Normal Uptake Values of Technetium-99m-Labeled Monoclonal Antigranulocyte Antibodies

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The aim of our study was to determine the normal range of the ^{99m}Tc -labeled anti-NCA 95 antigranulocyte antibody (AGAb) uptake in the bone marrow using the sacroiliac-to-background uptake ratio in the posterior view. **Methods:** We made 169 planar bone marrow scans on 162 patients who were each injected with 555 MBq AGAb. Fifty patients with the diagnosis of infection/pyrexia of unknown origin (PUO) and with completely normal bone marrow scintigraphy were defined as the normal group. Uptake ratios were calculated drawing irregular regions of interest around the sacroiliac and a background area, respectively. **Results:** The normal group revealed a mean uptake ratio of 7.3 ± 2.3 (range 4.4–12.6). Similar uptake ratios were obtained in patients with the primary diagnosis of infection/PUO and bone marrow extension (7.4 ± 2.2 , range 4.2–11.7), suggesting that the bone marrow reacts on infection primarily by extension into the periphery, without any significant increase of the activity of the central hemopoietic/granulopoietic bone marrow. Mean uptake ratios also were not significantly different in patients with normal bone marrow scintigraphy and the primary diagnosis of solid malignant tumors, lymphomas and plasmacytomas, and in patients with focal lesions visible on bone marrow scintigraphy (soft tissue inflammation or cold lesions in the bone marrow but with normal sacroiliac regions). Mean uptake ratios in the normal group were significantly age related, amounting to 8.5 ± 1.8 , 7.5 ± 1.9 and 6.1 ± 2.0 in patients younger than 40 yr, between 40 and 59 yr, and 60 yr or older, respectively ($p = 0.0025$). The method revealed good inter- and intraobserver agreement with correlation coefficients of about $r = 0.90$ and $r = 0.95$, respectively. Inter- and intraobserver coefficients of variation were 6.6% and 4.6%, respectively. **Conclusion:** Determination of the bone marrow uptake ratio is simple and reproducible. The normal values established in this study were age dependent, which has to be considered when interpreting bone marrow uptake ratios. The presence of infection/PUO, solid malignant tumors, lymphomas and plasmacytomas does not seem to alter the AGAb uptake ratio significantly. The most important application of the quantitative analysis of bone marrow scintigraphy could be the diagnosis and follow-up of diseases with depression of the central hemopoietic activity.

Key Words: bone marrow scintigraphy; antigranulocyte antibodies; normal uptake

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The visualization of bone marrow function is possible with several radiopharmaceuticals for scintigraphy of the erythropoietic, reticuloendothelial and granulopoietic bone marrow, but most of the routinely used radiopharmaceuticals reveal some disadvantages in daily work. The drawbacks of the radioisotopes of iron are the high absorbed doses, poor image quality (^{59}Fe) and the short half-life of cyclotron-produced ^{52}Fe . The value of imaging with ^{99m}Tc -labeled colloid is diminished by

excessive hepatosplenic accumulation and poor visualization of the adjacent skeletal structures (1,2).

Immunoscintigraphy using ^{99m}Tc -labeled antigranulocyte antibodies (AGAb) directed against the nonspecific cross-reacting NCA-95 antigen has been found to be a suitable modality for bone marrow imaging because of the simplicity of its use and excellent image quality with a high target-to-background ratio. The binding of the AGAb to cells in the bone marrow is very rapid and extensive because of the high concentration of granulocytes, promyelocytes and myelocytes that all express the NCA-95 antigen (3,4). Recent results suggest that bone marrow scintigraphy is more effective than conventional bone scintigraphy for estimating the presence and spread of malignant bone marrow infiltration (5–8). This procedure also yields information about both the extension and function of the granulopoietic bone marrow at the same time (1,2). Munz et al. (9–11) introduced a classification of the scintigraphic bone marrow status that consists of: (a) estimation of the bone marrow distribution pattern; (b) identification of focal lesions (cold lesions and hot spots); and (c) calculation of the uptake ratio between the sacroiliac region (corrected for background activity) and the background. The sacroiliac region is considered representative for the hemopoietically active bone marrow, and normal uptake values for this region are available for ^{99m}Tc nanocolloid (2). To the best of our knowledge, there are only two reports mentioning the normal AGAb uptake in the sacroiliac region in a small number of subjects using diverging or not particularly specified approaches (12,13). The aim of our study was to determine normal values for AGAb uptake in the bone marrow and to investigate the possible influence of various pathological conditions on the uptake ratio and on the distribution of the granulopoietic bone marrow.

MATERIALS AND METHODS

Patients

We performed 169 studies in 162 patients (73 women, 89 men; median age 54 yr, range 3–85 yr). We studied 118 patients because of suspected infection and pyrexia of unknown origin (PUO), and immunoscintigraphy was performed in 44 patients for the detection/exclusion of bone marrow infiltration by malignant diseases (17 lymphomas, 7 plasmacytomas, 20 solid malignant tumors). Fifteen patients with cold lesions or hot spots in the sacroiliac region were excluded from the study (10 with infection/PUO, 2 with lymphoma, 1 with plasmacytoma, 2 with solid malignant tumors). Our scintigraphic data was compared with total white blood-cell counts, C-reactive protein levels and body temperature, all recorded within 3 days before bone marrow scintigraphy. White blood-cell counts $> 10 \text{ G/l}$ and/or C-reactive protein levels $> 10 \text{ mg/l}$ and/or body temperature $> 37^\circ\text{C}$ were considered as signs of acute infection.

All studies performed were divided into six groups according to

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TABLE 1
Patient Groups According to Primary Diagnoses and Scintigraphic Bone Marrow Status

Group	Primary diagnosis	Bone marrow status	Number
A	Infection/PUO	I*, 0† (normal)	50
B	Infection/PUO	II‡, 0 (moderate extension)	25
C	Infection/PUO	I or II, 0; inflammatory focus	40
D	Solid malignant tumor	I or II, 0	7
E	Lymphoma or plasmacytoma	I or II, 0	16
F	Solid malignant tumor, lymphoma or plasmacytoma	I or II, cold lesion(s)	16

*Normal bone marrow distribution.

†No focal cold or hot lesions in the bone marrow.

‡Moderate bone marrow extension.

the underlying pathology and the classification of the bone marrow status as introduced by Munz et al. (11) (Table 1). The normal group (A) consisted of patients with diagnosis of infection/PUO and with completely normal bone marrow scintigraphy, as defined by a homogeneous appearance of the bone marrow confined to the central skeleton and the proximal one-third of the humeri and femora (Type I, according to Munz et al. (11)) and no signs of pathological activity accumulation in soft tissues. Groups B and C also consisted of patients with diagnosis of infection/PUO, but with moderate bone marrow extension (Type II) in Group B, and with or without extension, but with inflammatory foci present in soft tissues (Group C). Patients with diagnoses of malignant diseases were divided into three groups according to the underlying diagnosis and presence or absence of focal lesions in the bone marrow. Thus Groups D and E consisted of patients with solid malignant tumors and with lymphomas or plasmacytomas, respectively, but without focal lesions (addition 0, according to Munz et al. (11)). Group F included patients with any malignant diseases and cold lesions present in the bone marrow.

Image Acquisition

Imaging was performed using a dual-head, large field-of-view gamma camera (Multispect 2, Siemens, Erlangen, Germany) coupled to a computer (Icon, Siemens, Erlangen, Germany). Approximately 5 hr after injection of 555 MBq ^{99m}Tc-labeled AGAb (0.5 mg of BW 250/183, intact antibody, IgG₁ isotype; Behringwerke AG, Marburg, Germany) anterior and posterior whole-body bone marrow scans were obtained in contour-finding mode, using 256 × 1024-pixel acquisition matrix, high-resolution collimators and a table speed of 10 cm/min.

Image Processing

The quantitative analysis of the bone marrow scintigraphy was performed in the posterior view. The bone marrow region-of-interest was drawn around the sacroiliac area and the background region between the left or right kidney, spine and pelvis (Fig. 1). Finally, the uptake ratio was calculated as:

UR =

$$\frac{\text{ROI bone marrow (counts/pixel)} - \text{ROI background (counts/pixel)}}{\text{ROI background (counts/pixel)}}$$

where UR is the uptake ratio.

To investigate the interobserver variability in calculating the uptake ratio, a subgroup of 30 scans was independently analyzed by three experienced nuclear physicians, and coefficients of linear regression and of variability were determined. Intraobserver variability was checked, analyzing the same subgroup three times on three different days independently and blinded to patient data.

Statistical Analysis

Comparative testing of more than two variables at a time was performed by the Kruskal-Wallis analysis of variance. Differences were considered significant if the respective probability values were less than 0.05. Testing of Kruskal-Wallis subgroups was conducted with the Mann-Whitney-U test with the significance level adjusted according to Bonferroni. A summary of statistics, including mean values, s.d. and minimal and maximal values was run on all datasets.

The chi-squared test was used for testing differences between the two groups of patients with and without signs of acute infection and with normal bone marrow scintigraphy. Inter- and intraobserver agreement and variability were examined by linear regression and coefficients of variation (CV).

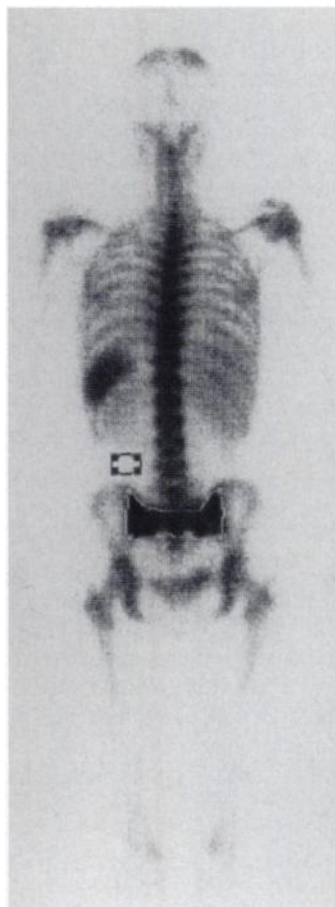


FIGURE 1. Normal AGAb bone marrow scan in the posterior view with the region of interest placed over the sacroiliac area and the background region between the left kidney, spine and pelvis.

TABLE 2

Mean Uptake Ratio Values, Standard Deviations, Ranges and Median Ages in Various Groups of Patients According to the Primary Diagnoses and Bone Marrow Status*

Group	UR**	s.d.	Range	Median age	Number
A	7.3	2.3	4.4-12.6	53	50
B	7.4	2.2	4.2-11.7	56	25
C	6.8	1.9	3.1-11.2	51	40
D	5.7	1.5	4.2-8.5	55	7
E	7.6	1.8	3.5-10.0	47	16
F	7.7	2.9	2.2-14.0	51	16

*Kruskal-Wallis, n.s., $p > 0.10$.

**Uptake ratio.

RESULTS

The patients with suspected infection/PUO and normal bone marrow scintigraphy were divided into two groups according to the presence or absence of clinical signs of acute infection. The mean uptake ratio value of the 19 patients without clinical signs of acute infection was not significantly different from the group of 31 patients with signs of acute infection present (6.8 ± 2.0 versus 7.5 ± 2.1 ; $p > 0.40$).

The mean uptake ratio value and s.d. for each of the six patient groups are presented in Table 2. In Group B, bone marrow extension was invariably moderate (extending to the distal parts of the femora and/or humeri).

To investigate the influence of age on AGAb uptake in the bone marrow, the patients from Group A were further divided into three age subgroups, as shown in Table 3. Uptake ratio declined with age, as verified by the Kruskal-Wallis analysis of variance ($p = 0.0025$). The uptake ratio of patients 60 yr or older was significantly lower than in both of the younger age groups.

The influence of the state of acute infection on the distribution of granulopoietic bone marrow was examined in patients who had bone marrow scintigraphy for detecting inflammatory foci but were negative (Groups A and B). Out of the patients from Groups A and B with clinical signs of acute infection present, 39% had a moderate bone marrow extension beyond the proximal one-third of the femora and/or humeri. On the other hand, only 14% of the patients from Groups A and B without any clinical signs of acute infection revealed a moderate degree of bone marrow extension. However, this difference did not reach statistical significance ($\chi^2 = 2.81$; threshold value = 3.84).

Interobserver agreement between D.H. and V.I. was $r = 0.84$ (0.93), between D.H. and W.-S.R. $r = 0.86$ (0.89), and between V.I. and W.-S.R. $r = 0.85$ (0.88). Interobserver variability amounted to $CV = 7.8\%$ (6.6%). The figures in parentheses

TABLE 3

Mean Uptake Ratio Values, Standard Deviations and Ranges, According to Age, in Patients with the Primary Diagnosis of Infection/PUO and Normal Bone Marrow Status (Group A)

Age (yr)	UR*	s.d.	Range	Number
<40	8.5 [†]	1.8	6.4-12.6	11
40-59	7.5 [‡]	1.9	5.0-11.6	22
≥60	6.1	2.0	4.4-11.0	17

*Uptake ratio.

[†] $p = 0.003$.

[‡] $p = 0.008$ vs. oldest age group (Mann-Whitney-U test, Bonferroni correction).

represent the respective data after excluding two patients with marked scoliosis of the spine (interobserver CVs 22% and 26%, respectively). Intraobserver agreement of the three readings was: $r = 0.94$ (0.94), $r = 0.93$ (0.93), $r = 0.97$ (0.98), for readings 1 and 2, 1 and 3 and 2 and 3, respectively. Intraobserver variability amounted to $CV = 4.7\%$ (4.6%).

DISCUSSION

AGAb uptake in the bone marrow reflects the degree of granulopoiesis, and the quantitative analysis of this uptake might be a valuable indicator of the granulopoietic bone marrow capacity and, since in most cases granulopoiesis parallels the hemopoietic activity, of the overall hemopoietic capacity (14). A quantitative analysis of bone marrow scintigraphy might be useful in several hematological disorders, like aplastic anemia, myelodysplastic syndrome, myelofibrosis, polycythemia vera and in the estimation of bone marrow recovery after therapy (1,2,15).

As a constitutive part of the evaluation of bone marrow status by ^{99m}Tc nanocolloid, Munz et al. (2,9,11) have introduced the bone marrow uptake index, which was obtained over the sacroiliac region and had a normal range between 2.66 and 4.24. Since the nanocolloid uptake in the bone marrow amounting to 15%-20% of the injected dose is much lower than the uptake of AGAb (55%, 4 hr after injection) (16), it was postulated that normal values of AGAb uptake ratio in bone marrow scintigraphy should be higher (6).

The technique of drawing the regions of interest and calculating the uptake ratio is simple and very reproducible. The sacroiliac region is sizeable and, thus, insensitive towards minor differences in drawing its contour. There is only a more or less thin layer of attenuating soft tissue between the sacroiliac region and the detector in the posterior view, thus minimizing the depth effect in planar scintigraphy. For the background region, care must be taken not to include parts of the kidney or bone marrow of ribs, spine or the iliac crest. There is no data in the literature concerning the reproducibility of this method by using AGAb. In our study the reproducibility of calculating the uptake ratio, as determined by the inter- and intraobserver agreement and variability, was found to be very high. In the case of marked scoliosis of the spine, however, reproducibility may be lower, most probably related to variations in the background regions.

As the best possible choice for a normal population, we considered the patients with completely normal bone marrow scintigraphy (bone marrow status I, 0; i.e., normal bone marrow distribution pattern, no focal lesions) and the primary diagnosis of infection/PUO but without any clinical signs of acute infection present. Since the uptake ratio in this group was not significantly different from the group of patients with normal bone marrow scintigraphy and suspected infection/PUO but with at least one of the clinical parameters abnormal, all the patients of both of these groups were pooled to form Group A, which served as our final group for the determination of normal uptake ratios. There was a clear-cut decline of uptake ratio with age confirming the observations of conversion of red to yellow bone marrow and decrease of blood flow through the bone marrow with advancing age (17,18). It is important, therefore, to differentiate normal uptake ratio values according to age. In our group of patients, it seemed reasonable to define normal values for subjects younger than 40 yr, for those between 40 and 59 yr and for those 60 yr or older. A more detailed analysis of the age dependence of the uptake ratio would require larger groups of subjects.

Bathmann et al. (12) described normal uptake ratio values

between 3.5 and 7.6 using the same approach in a control group of 20 patients with suspected inflammation. Since no further data on the scintigraphic appearance of the bone marrow or clinical data and especially age structure is given, we cannot validly comment on the differences in uptake ratio, mostly in the upper range, except for the different sizes of the subject groups. In the study performed by Chung et al. (13), there were 21 patients with malignant diseases serving as a control group. No further details are given on this patient group or on their scintigraphic presentation. The authors used a different anti-granulocyte antibody and a different technical approach to the calculation of the uptake ratio applying the lumbar spine, ilium and background region without detailed description. For these reasons no direct comparison to our results is possible.

The distribution of the hemopoietic bone marrow is age dependent. In neonates almost the entire bone marrow is hemopoietic, while in adults it is normally confined to the central skeleton (skull, sternum, vertebrae, ribs, pelvis) and to the proximal one-third of the humeri and femora (1,2,17). It is well known that fever and inflammation may cause an extension of the hemopoietic bone marrow into the periphery of the skeleton (1,2,19). There was a trend, although not statistically significant, towards a higher proportion of bone marrow extension in our patients with clinical signs of infection as compared to those without clinical signs. This extension, however, is not accompanied by a significant increase in activity of the central hemopoietic marrow, as suggested by the equal uptake ratios of our Groups A and B; in other words, in patients without and with marrow extension.

From the rather equal uptake ratios in patients with infection/PUO, solid malignant tumors and lymphomas or plasmacytomas it can be concluded that these diseases, at least as far as major bone marrow involvement is absent, do not cause significant alterations of the activity of the central bone marrow but may cause mostly moderate marrow extension. This means that the clinical value of the bone marrow uptake ratio might primarily consist of diagnosing and follow-up of diseases with potential bone marrow depression and monitoring of therapy effects. Unfortunately, there were no such diseases in our patient group to corroborate this statement. Thus, the question of whether or not the apparently wide normal ranges of bone marrow uptake allow a reliable distinction between normal and diminished uptake remains unanswered. However, a prospective study on the clinical value of the AGAb uptake in the initial diagnostic workup of diseases with depression of the bone marrow is being conducted.

CONCLUSION

The determination of the AGAb uptake ratio in the bone marrow is simple and reproducible. The normal uptake ratio is

age dependent, which must be considered when defining normal ranges of uptake ratios.

Infection/PUO, solid malignant tumors, lymphomas and plasmacytomas do not seem to alter the AGAb uptake ratio significantly. The most important applications of the quantitative analysis of bone marrow scintigraphy could be the diagnosis and follow-up of diseases with depression of the central hemopoietic activity.

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