Quantitative Assessment of Bone Marrow Cellularity by Magnetic Resonance Imaging in Workers with Long-Term Exposure to Solvents

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Abstract: This study was conducted to develop a noninvasive method of bone marrow cellularity evaluation in solvent-exposed painters. Six painters with hypocellular marrow and 132 referents were examined using magnetic resonance imaging (MRI). Full examination of the peripheral blood and bone marrow biopsy was done on each patient. Signal indices were calculated from the signal intensities measured at the vertebral bodies from T12 to S1 and on the paraspinal muscles on both the T1- and the T2-weighted image (T1WI and T2WI). Bone marrow cellularities of the painters were between 20.3% and 33.6%. Signal indices at T1WI were greater in the hypocellular marrow cases compared to those of the referents (p<0.05, p<0.01) and were significantly higher in older women compared with men (p<0.05). After adjusting for age and gender, the signal index of cases at S1 of T1WI was higher than that of the referents by 0.364. Five of the six cases had signal index at S1 of T1WI higher than the mean + 1 standard deviation for the same age group and gender. MRI signal indices can be used as a useful indicator of bone marrow cellularity in a high-risk population after adequate adjustment.

Key words: Magnetic resonance imaging, Bone marrow disorders, Leukopenia, Solvents, Occupational exposure

Introduction

Occupational hematologic disorders have been greatly decreased since the ban of benzene as a solvent after its toxicity to hematopoietic system had been known1). However, there are reports of different types of hematologic disorders as the amount of the industrial use of solvent increasing and as introduction of new solvents the toxicity of which was not fully evaluated yet. In Korea, aplastic anemia related to the exposure to 2-bromopropane2) and leukemia due to exposure to benzene in petrochemical company3) was recently reported.
The authors have reported cases of hypocellular marrow developed in shipyard painters after long-term exposure to ethyleneglycol monomethyl acetate (EGMME). EGMME is a hematotoxic solvent, which is widely used in the workplace. The main feature of this disorder was isolated leukopenia of mild to moderate degree, mainly due to neutropenia, in peripheral blood combined with prominent hypocellularity of bone marrow. In this disorder, severe cases of bone marrow disorders presenting as severe pancytopenia are very rare. However, mild cases of bone marrow disorders are hard to be detected because peripheral blood findings are not helpful in predicting the bone marrow damage in these cases.

Examination of the peripheral blood is a routine method for the screening of hematologic disorders, but it does not reflect the cellularity of the bone marrow directly. Such a discrepancy between peripheral blood and bone marrow finding has raised a need for the evaluation of other painters who has been exposed to the same solvent over a decade. However, use of bone marrow biopsy as a diagnostic tool only in cases of severe hematologic abnormality in peripheral blood makes effective evaluation of these cases difficult. Invasive nature of the bone marrow biopsy also poses a limitation in studying workers exposed to potential bone marrow toxicants and normal controls. Therefore, it is strongly needed to develop a noninvasive and effective tool that can measure the cellularity of bone marrow not only in diseased persons, but also in the normal population.

Development of magnetic resonance imaging (MRI) made it possible to assess the bone marrow cellularity noninvasively. MRI can provide qualitative and quantitative information on the cellularity of bone marrow by its property to discriminate the proportion of the fat and hematopoietic tissues. However, the use of MRI for the assessment of marrow cellularity was not applied to workers exposed to occupational exposure to substances that can cause bone marrow disorders. In occupational exposure, large number of workers are exposed to potential bone marrow toxicants without evident changes in peripheral blood until it reaches an advanced, potentially less reversible, stage. Use of noninvasive imaging technologies such as MRI could be used for the assessment of bone marrow cellularity among workers exposed to organic solvents potentially toxic to bone marrow. The authors conducted this study to develop practical indices of bone marrow cellularity by MRI and to develop reference values for the indices among normal population of various ages and genders.

### Materials and Methods

#### Subjects

Subjects were six painters (five males and one female, mean age 46.5 yr old, range 34–55 yr old) who have been diagnosed as hypocellular marrow by bone marrow biopsy between Aug. 1998 and Jan. 2000 after long-term exposure to organic solvents in a shipyard in Ulsan, Korea. Reference group was selected from those who have taken lumbar MRI using the same instrument at the same hospital. Among 421 subjects with age between 30 and 59 yr old, 25 subjects were randomly sampled from each age group of 10-yr interval in each gender. Among 150 subjects selected, only 132 subjects with an adequate quality of image and without any bone or bone marrow disorders that can influence the hematopoietic system were accepted as a reference group. Age distribution of the reference group was slightly lower than the patients, but not statistically significant (p>0.05, Table 1).

#### Methods

I. Hematologic evaluations and measurement of bone marrow cellularity

Venous blood was drawn from antebrachial vein of each patient. Complete blood cell count and peripheral blood smear was done during admission. Bone marrow aspiration and trephine biopsy was performed under local anesthesia on the posterior superior iliac crest by two hematologists (KUP and HJY). Marrow aspirate was smeared on the glass slide and stained with Wright after dried. Biopsy specimen was fixed in the 10% buffered formalin, then dehydrated and embedded in paraffin. Sections (4 µm) were routinely stained with hematoxylin and eosin. The specimens were examined under the light microscopy with magnifications of 200 and 400. Marrow cellularity was measured using image analyzer (Image-pro Plus ver 3.0 for Windows, Media Cybernetics Co., Silver Spring, MD, U.S.A.). The cellularity of bone marrow was measured at least two or more sites.

#### Table 1. General characteristics of the cases and the referents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Hypocellular marrow cases (N=6)</th>
<th>Referents (N=132)</th>
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<tbody>
<tr>
<td></td>
<td>No (%)</td>
<td>No (%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
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</tr>
<tr>
<td>30–39</td>
<td>1 (16.7)</td>
<td>50 (37.9)</td>
</tr>
<tr>
<td>40–49</td>
<td>3 (50.0)</td>
<td>50 (37.9)</td>
</tr>
<tr>
<td>50–59</td>
<td>2 (33.3)</td>
<td>32 (23.2)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (83.3)</td>
<td>67 (50.8)</td>
</tr>
<tr>
<td>Female</td>
<td>1 (16.7)</td>
<td>65 (49.2)</td>
</tr>
</tbody>
</table>
from stained sections and mean value of total areas was used as a cellularity for each sample. Each patient had at least three or more serial examinations of his/her bone marrow performed during their follow up period. Among these specimens, only that taken at the time closest to MRI were used as a cellularity for each subject.

II. Measurement of signal intensity from MRI

Signal intensity of bone marrow was measured at the sagittal image of lumbosacral vertebrae from both the patients and the referents. Both spin echo T1-weighted image (T1WI, TR 615 msec/TE 12 msec, FOV=17.5 × 28 ST 4 mm, matrix 150 × 256, Nex=4) and high spin echo T2-weighted image (T2WI, TR 4000 msec/TE 128 msec, FOV=28 × 28, ST=4 mm, matrix 276 × 512, Nex=4) was taken with the 1.5 Tesla superconduction system (Magnetom Vision Plus, Siemens, Germany). Signal intensity was measured at each T1WI and T2WI of vertebral bodies from T12 to S1. Mean signal intensity within a round region of interest (ROI) of 0.3–4.2 cm² size was measured at parasagittal sections of each vertebral body, two or three sections apart from midsagittal view, to avoid highly vascular area. The vertebrae with any radiologic pathology including compression fracture, deformities, or tumor were excluded from measurement. Signal intensity of reference region was measured at paraspinal muscles of each subject, at the area with highest muscle density and signal homogeneity, using round ROI at the same section (Fig. 1). Bone marrow signal index of each vertebral body was calculated by diving the signal intensity of each vertebral body by that of paraspinal muscle and multiplied by 100. This yielded 14 different signal indices for each subject. All the measurements of MRI signal intensities were performed by a radiologist (DSC) without providing information on the status of the subjects.

III. Data processing

Each variable was tested for the normality. Signal intensities between groups were compared by Student’s t-test and one-way ANOVA with Tukey test as a post hoc test for multiple comparisons, depending on the number of groups. Levene statistics was used to test the homogeneity of variance between groups. To identify the factors related to the signal index of the bone marrow, multiple linear regression analysis was done with signal index of bone marrow as a dependent variable. Reference values of bone marrow signal indices for each 10-yr age group of each gender were calculated using mean and 95 percent distribution of the variable. All the statistical process was done using SPSS/Win ver 10.0.

Results

Hematologic features of the painters with hypocellular marrow

Hypocellular marrow patients were between 34 and 55 yr old, with a work history as a shipyard painter in the company for 11 to 16 yr (Table 2). A male and a female painter had anemia. Leukocyte count of all the patients was equal or less than 4,000/mm³ except one, whose previous hematologic examination was also in the leukopenic range. Platelet count was below 140,000/mm³ in four of six patients, three of whom were below 70,000/mm³. In peripheral blood smears, however, there were only slight decrease in the leukocyte count. Bone marrow aspiration and biopsy of all the patients showed mild to moderate hypocellular marrow. A female patient showed iron deficiency anemia and another male patient exhibited decreased M/E ratio. Average cellularity of bone marrow, measured by image analyzer, was decreased in all the cases, between 20.3% and 33.6% (Fig. 2).
Comparison of bone marrow signal indices between patients and referents

Signal index of bone marrow at T1WI was lowest at L4 level, showing a bow-shaped distribution (Table 3). Signal indices of the patients were higher than those of the referents in all levels of vertebral bodies \((p<0.05, p<0.01)\). MRIs of the hypocellular marrow cases suggested more heterogeneous or patchy distribution of the marrow cellularity. However,
Levene statistics was not significant between two groups ($p>0.05$). Signal indices of T2WI showed a similar pattern along the level of vertebra, but the difference between the patients and the referents was not significant ($p>0.05$).

**Distribution of bone marrow signal indices in referents**

I. Bone marrow signal indices by age group and gender

Bone marrow signal indices were not significantly different between genders in referents ($p>0.05$, Fig. 3). The signal indices were significantly higher in female with age over 50 and more compared to those with age between 40 and 49, and those with age between 30 and 39 ($p<0.01$, $p<0.05$). However, age dependent difference was not significant among male referents ($p>0.05$).

II. Factors related to the bone marrow signal indices

Multiple regression showed that age and presence of hypocellular marrow were significant predictors of the signal indices, which is more prominent in lower lumbar vertebra and sacrum (Table 4, Fig. 4). Signal index of T1WI was higher 0.364 in patients at sacrum and 0.221 at L5 level.

**Choice of the optimum bone marrow signal index**

The authors plotted standardized signal indices of T1WI of the hypocellular marrow cases at each vertebral level after converting the raw values to those of the mean and the standard deviation of the signal indices of the same age and gender group (Fig. 5). Distribution of the signal indices were varied, but mostly distributed above mean value of the same age and gender group in five of the six cases. At the level of T12 and L4, distribution of the signal indices was above mean + 1 standard deviation in three of the six cases. However, at the level of L5 and S1, they were above mean + 1 standard deviation in five out of six cases. Therefore, T1WI signal index at S1 provided the best discrimination between the cases and the referents.

### Table 3. Comparison of MR signal indices of T1-weighted image between the hypocellular marrow cases and the referents (Mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Hypocellular marrow cases (N=6)</th>
<th>Referents (N=132)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T12</td>
<td>1.21 ± 0.21</td>
<td>0.95 ± 0.19</td>
<td>0.002</td>
</tr>
<tr>
<td>L1</td>
<td>1.12 ± 0.22</td>
<td>0.93 ± 0.17</td>
<td>0.009</td>
</tr>
<tr>
<td>L2</td>
<td>1.05 ± 0.26</td>
<td>0.88 ± 0.16</td>
<td>0.018</td>
</tr>
<tr>
<td>L3</td>
<td>1.01 ± 0.24</td>
<td>0.85 ± 0.15</td>
<td>0.016</td>
</tr>
<tr>
<td>L4</td>
<td>0.98 ± 0.22</td>
<td>0.83 ± 0.15</td>
<td>0.02</td>
</tr>
<tr>
<td>L5</td>
<td>1.15 ± 0.18</td>
<td>0.86 ± 0.17</td>
<td>0.000</td>
</tr>
<tr>
<td>S1</td>
<td>1.44 ± 0.25</td>
<td>1.03 ± 0.26</td>
<td>0.000</td>
</tr>
</tbody>
</table>

**Fig. 3.** MR indices at T1-weighted image by age group and gender. Effect of age in the signal intensity is not significantly different in males (A), but evident in females (B) after age 50 ($p<0.05$, $p<0.01$). Signal indices (C) are not significantly different by gender.
Range of mean ± 1 SD of the signal index at T1WI at the age group 30 and 39, 40 and 49, and 50 and 59 were 1.187, 1.242, and 1.216 in male, and 0.989, 1.202, and 1.687 in female; that of mean ± 2 SD at the age group 30 and 39, 40 and 49, and 50 and 59 were 1.369, 1.512, and 1.373 in male and 1.115, 1.395, and 2.082 in female.

**Discussion**

MRI was applied for the assessment of bone marrow cellularity since its early time of development\(^{14-16}\). Bone marrow consists of mostly hematopoietic cells and fat cells filling the available space. Bone marrow cellularity is a proportion of hematopoietic cells among total marrow space,
Fig. 5. Relative distribution of the muscle signal indices at T1WI, compared to the mean and standard deviation of the referents of the same age and gender. Hypocellular cases are distributed over wide range in lumbar vertebrae, but clustered at T12, L5, and S1. At L5 and S1 level, five out of six cases are distributed over one standard deviation.

reflecting the ratio between hematopoietic cells and fat cells. Hematopoietic cells and fat cells have different composition of fat, water and protein, each of which has different characteristic on magnetic resonance imaging. Cellularity of bone marrow can be measured from T1- or T2-weighted images of usual spin echo sequence. T1WI, having good contrast ratio for hematopoietic and fat cells, was utilized for the qualitative and quantitative assessment of cellularity of bone marrow from early stage of development of MRI17). MRI is a noninvasive technique and can be used for the repetitive study without definite health risk. It has advantages in its ability to measure cellularity at multiple sites, and in assessment of cellularity even in case of heterogeneous distribution. However, MRI is a costly examination, and other lesions causing similar signal changes should be differentiated. It is not suitable to be used for the evaluation of the cellularity at bones with narrow marrow space like pelvic bones, which is the usual site for the bone marrow biopsy. Vertebral body, with its rich red marrow and rectangular area, provides the best condition for the MRI measurement of bone marrow cellularity. So far, MRI is mainly used for the follow up examination of the patients with bone marrow disorders. However, this study showed that MRI could be used for the assessment of the patients with mild to moderate bone marrow depression with minimal or no hematologic abnormalities.

This study showed that signal indices are higher in hypocellular marrow patients compared to the referents, but distribution of the indices were wider in the patients than the referents, i.e., the patients had higher variance compared to the referents, although the difference was not significant due to small sample size. Radiologic features of the bone marrow of the patients showed more heterogeneous and patchy distribution of the signal intensity compared to that of the referents. This is compatible with a report that regeneration of the hematopoietic cells in bone marrow expands from islands of regenerative centers18, 19). Distribution of the signal indices among the referents showed a characteristic pattern simulating the curvature of thoracolumbosacral spine, i.e., high at lower thoracic spine, lowering as it comes along the lumbar spine, lowest at L4, then gets higher up to sacrum, highest at S1. Richards et al.20) also reported that T1 relaxation time was shorter at lower lumbar spines that at the upper ones. Concordance of the signal indices with the spinal curvature may be a result of the biased measurement of signal intensity due to varying distance of each vertebra from the detectors. To reduce such deformity of signals, it is preferable to place the regions of
interest closer to the center of the image. However, at the ordinary MRI images, sacrum and upper lumbar vertebrae would have to be placed at the periphery of the images for both of the regions to be arranged at the same image. Taking an additional image of the sacrum can be more informative as well as making less deformity of the signals, but taking such an additional images from the referents will need a special referent group, making it difficult to select referents from ordinary hospital patients taking lumbar MRI. Therefore, the authors have used the ordinary sagittal view of thoraco-lumbo-sacral spine for the measurement of signal intensities in spite of its potential distortion of signals.

Signal indices increase with age among the referents and this trend was more prominent in women after fifty (Fig. 3). This phenomenon can be primarily related to the age-dependent decrease of bone marrow cellularity\textsuperscript{14, 20}. Prominent increase of signal indices in postmenopausal women may be related to the decreased bone density and/or increased fat content, because T1 signal intensity of the bone marrow is affected by bone density and high calcium or iron deposition in bone matrix\textsuperscript{21–23}. The authors have used signal indices based on the muscle signal intensity as a reference area, to compare the signal intensities obtained from different individuals and views each of which has different conditions of obtaining signal intensities. Subcutaneous fat area is frequently used for this purpose\textsuperscript{24, 25}, but compared to Caucasians, Asians used to have less subcutaneous fat deposit for the stable signal to be obtained. Lowest possible level of signal intensity measurement for the spinal cord was at T12 level. Peripheral location of the level of lowest spinal cord, at the level of T12, in the lumbosacral image makes it prone to image distortion. In this study, we have evaluated the signal indices based on subcutaneous fat and spinal cord as well as on the muscles. Signal intensities from the subcutaneous fat or spinal cord showed wide variation between measurements and resulting signal indices did not have good differentiation between the patients and the referents (data not shown).

Assessment of cellularity using signal indices measurement has several limitations. The measurements taken from different instruments cannot be compared between each other, and needs its own referents in each instrument. Direct measurement of T1 relaxation time can be a solution, but it needs additional images and time\textsuperscript{24, 26}. A fat suppression sequence, short tau inversion recovery (STIR), can be used for both quantitative and qualitative method of bone marrow cellularity\textsuperscript{27, 28}. In contrast to T1 relaxation time measurement, cellularity is assessed by directly measuring the water density after suppressing the fat signal. It takes additional time of several minutes for the measurement, but it does not need additional hardware. Several other techniques, chemical shift misregistration artifact\textsuperscript{29, 30}, inner volume spectroscopic CPMG image technique\textsuperscript{11}, or MR spectroscopy\textsuperscript{31–33}, can provide a good assessment of the bone marrow cellularity, with use of additional instruments.

In this study, we have provided practical indices for the assessment of the cellularity of bone marrow. Our method has advantage in that it can be measured from ordinary images of lumbar MRI without additional instruments or software. This made the assessment of bone marrow cellularity on the normal population easy by using the stored image. Although MRI assessment of bone marrow cellularity has been used widely in clinical medicine, it has not been applied in the field of occupational exposure. Because of small sample size, statistical analysis on the correlation between cellularity and signal indices, and on the difference between the patients and referents in this study had low statistical power. Additional study on the larger number of hypocellular marrow patients will provide better information on the application of the signal indices. In spite of several limitations, we believe that this method can be used for the early screening and follow up of the bone marrow disorders in the workers exposed to chemicals that are potentially toxic to hematopoietic system.

References

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MRI ASSESSMENT OF BONE MARROW CELLULARITY IN PAINTERS


