INTRODUCTION

In the absence of a known affected family member, frequent symptoms of Gaucher disease (GD), a rare lysosomal storage disorder, such as thrombocytopenia or splenomegaly, often lead to hematological diagnostic workup.

OBJECTIVES

The aim of the study was to compare the clinical utility of aspiration biopsy of the bone marrow (ASP) with trephine biopsy (TB) for the diagnosis of GD type 1 (GD1).

PATIENTS AND METHODS

Six non-Jewish patients with sporadic GD1 were initially examined with ASP and TB to establish the cause of cytopenia and splenomegaly. In the current study, samples from each patient consisted of 2 bone marrow slides. On each slide, 500 nucleated cells were counted and then averaged. The composition of bone marrow TBs was assessed using digital images analyzed on a computer.

RESULTS

Of 6 patients, 5 carried at least 1 N370S allele with a c.1226A>G mutation in the *GBA1* gene. The median number of Gaucher cells identified during cytological assessment of bone marrow smears was 4 (range, 1–18), and the median percentage of Gaucher cells was 0.4% (range, 0.1%–1.8%). The absolute proportion of Gaucher cells in histological samples ranged from 22% to 36% (median value, 28%), and the ratio of Gaucher cell infiltrate to hematopoietic tissue ranged from 34% to 54% (median value, 47%). The median value of the ratio of Gaucher cells to hematopoietic tissue was strikingly lower when using ASP compared with TB ($P = 0.028$).

CONCLUSIONS

Our results indicate that ASP is not a reliable diagnostic tool for the detection of GD1. Thus, patients with unclear long-lasting splenomegaly and/or thrombocytopenia, in whom bone marrow aspirate cytology is negative for Gaucher cells, should be routinely referred for an enzymatic assay for GD.

INTRODUCTION

Gaucher disease (GD) is a progressive, multisystem lysosomal storage disorder caused by the deficient activity of the lysosomal enzyme, glucocerebrosidase, resulting from autosomal recessive mutations in the *GBA1* gene (1q21). Common symptoms of GD type 1 (GD1), such as thrombocytopenia, anemia, or splenomegaly, often lead to hematological diagnostic workup. As part of such workup, routine diagnostic procedures include aspiration biopsy of bone marrow (ASP) and trephine biopsy (TB), for the cytological assessment of bone marrow smears (BM-S) and histological evaluation of bone marrow TBs (BM-TB), respectively.

The lipid-laden macrophages, referred to as storage or Gaucher cells, are the pathological hallmark of GD. These large cells of 20 to 100 µm in diameter with small, usually eccentrically placed nuclei, have slightly basophilic cytoplasm with characteristic crinkles or striations described as having a “wrinkled tissue paper” appearance. Gaucher cell infiltrates can be found in organs and
in Stockholm. Of these, 6 non-Jewish patients (2 women and 4 men) with sporadic GD1 who initially underwent diagnostic ASP and TB as part of the evaluation for cytopenia accompanied by splenomegaly were included in this analysis. Bone marrow samples were collected under local anesthesia from an entry site on the posterior iliac crest of patients in the prone position. The samples were stained with May–Grünwald–Giemsa (BM-S) or hematoxylin and eosin (BM-TB) stains according to routine methods. Stored bone marrow samples were reassessed by a hematopathologist for study purposes.

Differential counts of BM-S were made under ×400 magnification using an Olympus BX 40 microscope. Samples from each patient consisted of 2 slides. On each slide, 500 nucleated cells were counted and then averaged. An assessment of the composition of BM-TB, ie, a relative proportion of the hematopoietic tissue, Gaucher cells, fat tissue, and trabecular bone, was carried out using digital images and the GNU Image Manipulation Program (GIMP2) (freeware available at www.gimp.org).

tissues rich in cells of the mononuclear phagocyte system, such as the spleen, liver, and, in particular, the bone marrow.

The results of ASP and/or TB examinations often give the first clue towards GD diagnosis in non-Jewish patients who do not have any previously known GD1-affected family members (ie, sporadic GD1), since they disclose the presence of macrophages having a Gaucher cell appearance.2,4,5 However, because there are virtually no published studies comparing ASP and TB in the diagnostic workup of GD, little is known about the utility of these 2 methods in the assessment of bone marrow involvement in GD.

The aim of our study was to compare the results of cytological and histological analyses of BM-S and BM-TB, obtained by means of ASP and TB, in adult non-Jewish patients with newly diagnosed GD1.

**PATIENTS AND METHODS**

There are currently 35 patients diagnosed with GD1 in Sweden.7 Between 2002 and 2013, 16 adults with GD1 were followed at the Karolinska University Hospital in Stockholm. Of these, 6 non-Jewish patients (2 women and 4 men) with sporadic GD1 who initially underwent diagnostic ASP and TB as part of the evaluation for cytopenia accompanied by splenomegaly were included in this analysis.

Bone marrow samples were collected under local anesthesia from an entry site on the posterior iliac crest of patients in the prone position. The samples were stained with May–Grünwald–Giemsa (BM-S) or hematoxylin and eosin (BM-TB) stains according to routine methods. Stored bone marrow samples were reassessed by a hematopathologist for study purposes.

Differential counts of BM-S were made under ×400 magnification using an Olympus BX 40 microscope. Samples from each patient consisted of 2 slides. On each slide, 500 nucleated cells were counted and then averaged. An assessment of the composition of BM-TB, ie, a relative proportion of the hematopoietic tissue, Gaucher cells, fat tissue, and trabecular bone, was carried out using digital images and the GNU Image Manipulation Program (GIMP2) (freeware available at www.gimp.org).

**TABLE 1**

<table>
<thead>
<tr>
<th>Pt</th>
<th>Sex/age</th>
<th>GBA1 gene mutations (mutated alleles)</th>
<th>Age at symptom onset, y</th>
<th>SMG</th>
<th>SPC (age, y)</th>
<th>Bone disease</th>
<th>GBA</th>
<th>Chito</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/21</td>
<td>c.798C&gt;G/c.1040T&gt;G (F227I/I308S)</td>
<td>3</td>
<td>NA</td>
<td>5</td>
<td>yes (B)</td>
<td>0.49</td>
<td>9743</td>
</tr>
<tr>
<td>2</td>
<td>F/56</td>
<td>c.1226A&gt;G/c.1226A&gt;G (N370S/N370S)</td>
<td>55</td>
<td>yes</td>
<td>no</td>
<td>yes (A)</td>
<td>0.43</td>
<td>1549</td>
</tr>
<tr>
<td>3</td>
<td>M/65</td>
<td>c.1226A&gt;G/c.1448T&gt;C, c.1483G&gt;C, c.1497G&gt;C (N370S/L444P, A456P and V460V)</td>
<td>51</td>
<td>yes</td>
<td>no</td>
<td>yes (A)</td>
<td>0.1</td>
<td>1322</td>
</tr>
<tr>
<td>4</td>
<td>M/66</td>
<td>c.721G&gt;A/c.1226A&gt;G (G202R/N370S)</td>
<td>61</td>
<td>yes</td>
<td>no</td>
<td>yes (B)</td>
<td>0.41</td>
<td>2448</td>
</tr>
<tr>
<td>5</td>
<td>M/81</td>
<td>c.1226A&gt;G/c.1448T&gt;C (N370S/L444P)</td>
<td>30</td>
<td>NA</td>
<td>32</td>
<td>yes (A)</td>
<td>0.59</td>
<td>2170</td>
</tr>
<tr>
<td>6</td>
<td>M/84</td>
<td>c.1226A&gt;G/c.1448T&gt;C (N370S/L444P)</td>
<td>20</td>
<td>NA</td>
<td>22</td>
<td>yes (B)</td>
<td>0.32</td>
<td>2804</td>
</tr>
</tbody>
</table>


**FIGURE**

Gaucher disease type 1: cytological (A) and histological (B) images of bone marrow samples obtained by aspiration biopsy of the bone marrow and trephine biopsy; A – presence of cells from 2 hematopoietic series, including a centrally placed histiocyte with Gaucher cell morphology (May–Grünwald–Giemsa stain; magnification, ×400); B – hypercellular bone marrow with all hematopoietic series present, including prominent histiocytic infiltrates composed of Gaucher cells (hematoxylin and eosin stain; magnification, ×200).
TABLE 2 Cytological composition of bone marrow smears obtained by aspiration biopsy in patients with Gaucher disease type 1

<table>
<thead>
<tr>
<th>Pt</th>
<th>ErP</th>
<th>Bl</th>
<th>Pml</th>
<th>My</th>
<th>Neu</th>
<th>Eos</th>
<th>Bas</th>
<th>Mono</th>
<th>Lym</th>
<th>Plasm</th>
<th>Mgk</th>
<th>GCs</th>
<th>Total</th>
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<tbody>
<tr>
<td>1</td>
<td>260</td>
<td>5</td>
<td>9</td>
<td>94</td>
<td>313</td>
<td>12</td>
<td>2</td>
<td>25</td>
<td>279</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1000</td>
</tr>
<tr>
<td>2</td>
<td>335</td>
<td>15</td>
<td>22</td>
<td>53</td>
<td>428</td>
<td>7</td>
<td>3</td>
<td>71</td>
<td>62</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>1000</td>
</tr>
<tr>
<td>3</td>
<td>154</td>
<td>10</td>
<td>8</td>
<td>35</td>
<td>469</td>
<td>21</td>
<td>11</td>
<td>71</td>
<td>214</td>
<td>0</td>
<td>1</td>
<td>6</td>
<td>1000</td>
</tr>
<tr>
<td>4</td>
<td>259</td>
<td>9</td>
<td>17</td>
<td>124</td>
<td>401</td>
<td>32</td>
<td>1</td>
<td>28</td>
<td>114</td>
<td>0</td>
<td>0</td>
<td>15</td>
<td>1000</td>
</tr>
<tr>
<td>5</td>
<td>173</td>
<td>8</td>
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<td>7</td>
<td>560</td>
<td>7</td>
<td>4</td>
<td>62</td>
<td>124</td>
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<td>0</td>
<td>3</td>
<td>1000</td>
</tr>
<tr>
<td>6</td>
<td>355</td>
<td>8</td>
<td>22</td>
<td>71</td>
<td>300</td>
<td>12</td>
<td>0</td>
<td>83</td>
<td>121</td>
<td>10</td>
<td>0</td>
<td>18</td>
<td>1000</td>
</tr>
</tbody>
</table>

Reference values (per 1000 nucleated bone marrow cells) according to Sundström and Öst (1985): erythroblasts, 80–400; myeloblasts, 3–50; promyelocytes, 10–80; myelocytes and metamyelocytes, 10⁶–365; band granulocytes, 50–140; neutrophilic granulocytes, 70–300; eosinophilic granulocytes, 5–40; basophilic granulocytes, 0–7; monocytes, 5–50; lymphocytes, 30–170; plasma cells, 0–20; megakaryocytes, 0–20; promyelocytes, others – see TABLE 1.


TABLE 3 Histological composition of bone marrow in trephine biopsies in patients with Gaucher disease type 1

<table>
<thead>
<tr>
<th>Pt</th>
<th>Histological composition of the bone marrow, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HT</td>
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<tr>
<td>1</td>
<td>39</td>
</tr>
<tr>
<td>2</td>
<td>46</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>5</td>
<td>29</td>
</tr>
<tr>
<td>6</td>
<td>23</td>
</tr>
</tbody>
</table>

Abbreviations: HT – hematopoietic tissue, others – see TABLE 2.

TABLE 4 Mean reference values in healthy individuals, according to Burkhardt et al.³

<table>
<thead>
<tr>
<th>Age group, y</th>
<th>Hematopoietic tissue</th>
<th>Fatty tissue</th>
<th>Trabecular bone</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–39</td>
<td>47.5%</td>
<td>27.9%</td>
<td>21.4%</td>
</tr>
<tr>
<td>40–59</td>
<td>43.1%;*</td>
<td>33.8%</td>
<td>20.5%</td>
</tr>
<tr>
<td>60–99</td>
<td>43.8%</td>
<td>37.7%</td>
<td>15.3%</td>
</tr>
</tbody>
</table>

Reference values for the proportion of hematopoietic tissue to fat tissue according to Sundström and Öst: 25%–75% in adults; about 40% in the age group of 30–70 years. The hematopoietic tissue fraction decreases slightly after the age of 70 years.

The reference values for cytological and histological composition of bone marrow samples in healthy individuals were obtained from the studies of Burkhardt et al.³ and Sundström and Öst.⁴ The nonparametric Wilcoxon signed-rank test was used to evaluate statistically significant differences between the results of ASP and TB examinations for the detection of Gaucher cells (the relatively small sample analyzed could not be assumed to be normally distributed). The P value was 2-tailed and calculated using Stata 9.2 (StataCorp LP, College Station, TX, USA). A P value of less than 0.05 was considered statistically significant.

The study protocol was developed according to the ethical standards of the Declaration of Helsinki and approved by the local ethics committee in Stockholm. All patients provided their informed consent to participate in the study.

RESULTS The median age of the patients was 65 years (range, 21–84 years). Three patients (50%) had undergone splenectomy. All but 1 patient (83%) carried at least 1 N370S allele with the c.1226A>G mutation in the GBA1 gene. Patient characteristics are presented in TABLE 1.

Examples of cytological and histological images of bone marrow samples, obtained by ASP and TB from one of the patients, are shown in the FIGURE. The results of cytological analyses of BM-S including identified Gaucher cells are presented in TABLE 2. The median number of Gaucher cells identified in the studied patients was 4 (range 1–18), and the median percentage of Gaucher cells among all nucleated bone marrow cells was 0.4% (range, 0.1%–1.8%).

The proportions of hematopoietic tissue, Gaucher cells, fat tissue, and trabecular bone in BM-TB from the patients are presented in TABLES 3 and 4. The Gaucher cell burden in BM-TB ranged from 22% to 36% (median value, 28%) and the ratio of Gaucher cells to hematopoietic tissue ranged from 34% to 54% (median value, 47%). The ratio of Gaucher cells to hematopoietic tissue in ASP...
pseud Gaucher cells were also reported in many bone marrow specimens, liver biopsy, or a surgical procedure. In many countries (approximately the last 20 years), TB has been introduced into routine hematological diagnostic workup relatively recently in many countries (approximately the last 20 years), at the time when bone marrow examination was no longer recommended for the sole purpose of GD diagnosis.11

The results of the present study indicate a low sensitivity of ASP in detecting Gaucher cells in the bone marrow. Most analyzed patients (4 of 6) had 6 or fewer Gaucher cells among 1000 nucleated hematopoietic cells. Of note, routine differential count in BM-S usually includes only 200 nucleated hematopoietic cells. Thus, there is a serious risk of overlooking GD in some patients when using only ASP, and we conclude that ASP is not a reliable diagnostic tool for GD1.

Gaucher cells are often tightly packed in the affected areas of the bone marrow tissue and therefore difficult to aspirate by ASP, which may explain our findings. Additional problems with aspiration of Gaucher cells can result from increased density of reticulin fibers in the bone marrow. The sensitivity of TB for detection of Gaucher cells in the bone marrow is much higher, and the observed detected Gaucher cell burden in the present study was approximately 100-fold higher compared with cytological specimens obtained by ASP. Although histological findings are reliable in the diagnosis of GD1, TB is a more advanced procedure than ASP, and it should be performed by an experienced physician.

When an ASP sample is negative for Gaucher cells (false-negative ASP result) and an enzyme assay for GD is not performed, there is a serious threat that the diagnosis of GD1 may be postponed for many years.16 Therefore, proper interpretation and recognition of the limitations of ASP results are crucial in avoiding diagnostic delays in patients affected with this treatable condition.
The introduction of enzyme replacement therapy (ERT) for the treatment of GD1 has resulted in a dramatic improvement in the prognosis of the affected patients.\textsuperscript{1,2} ERT has the potential to positively affect all domains of GD1 (ie, hematological, visceral, and skeletal).\textsuperscript{23,24} Since 1991, GD has become a model for the development of ERT in other lysosomal storage disorders.\textsuperscript{25,26}

Conclusions Common initial symptoms of GD1, such as thrombocytopenia or splenomegaly, often result in patient referral to a hematologist for diagnostic workup. To obtain a reliable diagnosis of GD, cytohistological examinations are neither necessary nor sufficient. The gold standard for a definitive diagnosis of GD requires confirmation of reduced enzymatic activity of glucocerebrosidase in leukocytes, cultured fibroblasts, or amniocytes obtained during prenatal diagnosis.\textsuperscript{27} Therefore, enzymatic assays should be applied in suspected cases. Measurement of glucocerebrosidase is supplemented by the GBA1 mutation analysis.\textsuperscript{1-3}

However, since GD is a very rare condition and its clinical manifestation may mimic lymphoma or other hematological diseases, bone marrow or liver biopsy is usually performed before the diagnosis of GD is considered, especially in the absence of known affected family members.

To the best of our knowledge, this is the first report comparing clinical utility of ASP with that of TB in the diagnosis of sporadic cases of GD. The results indicate that ASP is not a reliable diagnostic tool for detecting Gaucher cells. Therefore, patients with unclear long-lasting splenomegaly and/or thrombocytopenia and in whom cytological assessment of BM-S provides negative results, should proceed routinely to enzymatic assays for GD.

Acknowledgements This work was supported by a grant provided by the Stockholm County Council (ALF project). We acknowledge Mrs. Pam Pickering for linguistic expertise.

Contribution statement MM conceived the idea for the study. MK and AM-K contributed to the design of the research. All authors were involved in data collection. SR and AM-K analyzed the data. MM coordinated funding for the project. All authors edited and approved the final version of the manuscript.

REFERENCES

Wartość kliniczna zastosowania różnych metod badania szpiku kostnego w diagnostyce osób dorosłych ze sporadyczną chorobą Gauchera typu 1

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SŁOWA KLUCZOWE
biopsja aspiracyjna, choroba Gauchera typu 1, komórka Gauchera, szpik kostny, trepanobiopsja

STRESZCZENIE

WProwadzenie W przypadku niestwierdzenia zachorowania w rodzinie, częste objawy choroby Gauchera (Gaucher disease – GD), rzadkiej lizosomalnej choroby spichrzeniowej, takie jak małopłytkowość lub splenomegalia, często prowadzą do wdrożenia diagnostyki hematologicznej.

CELE Celem badania było porównanie użyteczności klinicznej biopsji aspiracyjnej szpiku kostnego (aspiration biopsy – ASP) z trepanobiopsją (trephine biopsy – TB) w rozpoznawaniu GD typu 1 (GD1).

Pacjenci i metody Sześciu pacjentów ze sporadyczną GD1 i niebędących pochodzenia żydowskiego, zbadano początkowo przy użyciu rutynowej ASP i TB w celu ustalenia przyczyny małopłytkowości i powiększenia śledziony. W ramach obecnego badania, analizowany materiał od każdego pacjenta składał się z 2 szkiełek z rozmazami szpiku kostnego, gdzie na każdym z nich oceniano 500 komórek jądrzastych, a następnie liczono średnią. Ocenę składu szpiku kostnego uzyskanego metodą TB przeprowadzono komputerowo, analizując obrazy cyfrowe szpiku.

Wyniki U większości pacjentów (5/6) stwierdzono obecność co najmniej jednego allelu N370S z mutacją c.1226A>G w genie GBA1. Średnia liczba komórek Gauchera zidentyfikowanych podczas oceny cytologicznej rozmazów szpiku kostnego wyniosła 4 (zakres 1–18), a średni odsetek komórek Gauchera wyniósł 0,4% (zakres 0,1–1,8%). Całkowity odsetek komórek Gauchera stwierdzanych w preparatach histologicznych wahal się od 22% do 36% (mediana 28%), a stosunek nacieków z komórek Gauchera do tkanki krwiotwórczej mieścił się w zakresie 34–54% (mediana 47%). Wartość mediany stwierdzanej odsetka komórek Gauchera w stosunku do tkanki krwiotwórczej była wyraźnie mniejsza przy użyciu do badania szpiku kostnego ASP w porównaniu z TB (p = 0,028).

Wnioski Uzyskane wyniki wskazują, że ASP nie jest dostatecznie wiarygodną metodą diagnostyczną wykrywania GD1. Dlatego pacjentów z niejasnym długotrwałym powiększeniem śledziony i/lub małopłytkością, u których wynik badania cytologicznego na obecność komórek Gauchera materiału uzyskanego za pomocą ASP jest ujemny, powinno się rutynowo kierować do diagnostyki enzymatycznej choroby Gauchera.