STERNAL MARROW PUNCTURE IN CHILDREN

BY

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In certain blood dyscrasias the peripheral blood gives a poor indication of the pathological changes in the bone marrow, and a study of the actual nature and condition of the marrow is essential in establishing the final diagnosis of the disease. The choice of the sternum for this procedure sounds logical owing to its superficial location, the thin anterior lamina over the marrow cavity and its lifelong activity.

It is only during the past thirty years that bone marrow has been employed during life as a means of diagnosis. Ghedini in 1908, choosing the tibia as the site for biopsy, selected patients with latent malaria and leishmaniasis, and observed that the parasites were present in the bone marrow but absent from the peripheral blood. This method was, however, of no value in demonstrating the changes in the bone marrow of older children and adults, since the tibial marrow is only active from birth until the age of seven years, at which age it begins to undergo fatty metamorphosis, so that by the age of fifteen it is completely inactive. Seyfarth (1923) selected the sternum in place of the tibia for marrow biopsy, but still used trepanning, which being a surgical procedure was not suitable for routine clinical use. In 1929 Arinkin introduced the aspiration method for obtaining sternal marrow, and in 1937 Kato, dealing exclusively with infants and children, attempted to establish the normal picture of bone marrow at various ages. In 1939 Vogel and Bassen reported their observations on the marrow of a group of normal infants and children and on an unselected group with blood dyscrasia and other diseases.

Developmental anatomy of the human sternum

A post-mortem study, including radiological examination, of a series of sterna at various ages, including those of six and seven months foetuses, was undertaken to determine the developmental stages of the human sternum and to find out the correct depth to which the needle should be plunged to reach the marrow lacunae. The conclusions arrived at may be summarized as follows:

1. The ossification centres of the sternum, which correspond to the red marrow, appear at the sixth month of intra-uterine life, first in the manubrium, then in the other sternebrae.

2. Two stages of development of ossification centres have been noted. In the first, that of 'pairing,' one centre appears on either side of the middle line. At birth those of the manubrium are fused into one
large centre, while the next three segments contain small paired centres which are separate from each other.

3. In the second stage the paired centres in each segment fuse into one larger one, a process usually completed between the sixth and tenth years, when each sternebra becomes almost completely filled with its ossification centre corresponding to the bone-marrow content. In the lower segment of the sternum, however, pairing may persist as late as the twelfth year (fig. 1).

4. The entire sternum is enclosed in a firm periosteal covering, the perforation of which is painful, making it advisable to anaesthetize this tissue by injecting some novocain subperiosteally when performing the sternal puncture.

5. The cartilaginous partitions between the sternebrae explain the failure of sternal puncture performed opposite a rib. It should be done opposite an interspace, otherwise the needle will hit against cartilage.

6. In the adult, however, the partitions in the body of the sternum are absent, and the marrow forms one continuous lake, and is equally accessible opposite the ribs and interspaces. The ossification centre of the xiphisternum, which is absent in children, starts to appear in adult life (fig. 2).

### Technique of sternal puncture

#### General considerations

1. The centres of the manubrium and first sternebra are the earliest to appear; they fuse and attain a moderate size as early as the sixth month of life, and so appear more suitable than the other segments of the sternum for
Fig. 2.—Longitudinal sections to demonstrate the anatomical differences between the sternum of a child (left) and that of an adult (right).

performing the sternal puncture. Puncturing the manubrium, however, is not free from risk owing to the big vessels that lie behind, and so the centre opposite the second intercostal space appears the most suitable for this purpose in children. In the first few months of life this centre may be poorly developed and the puncture has to be made in the manubrium.

2. The anterior lamina of the sternum is cartilaginous in infants and young children, and gets gradually ossified in later childhood and adult life. In the case of the bony anterior lamina, a sense of resistance is experienced while performing the puncture, which suddenly disappears when the needle enters the marrow cavity. If the anterior lamina is still cartilaginous it is very difficult to know when one has reached the marrow through the soft cartilage, as in this case there is neither resistance nor a sense of 'giving way'; it is therefore necessary to be careful as regards the depth the needle has to attain in order to reach the marrow cavity, since otherwise there is the possibility of forcing the needle too deeply into the mediastinal space.

3. The total thickness of the sternum is composed of the anterior and posterior laminae and the marrow space between them. The dimensions at
the level of the second interspace in the children of our series whose ages varied from a few days to ten years were as follows:

<table>
<thead>
<tr>
<th></th>
<th>MINIMUM</th>
<th>MAXIMUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total thickness of the sternum</td>
<td>4-5 mm.</td>
<td>7-5 mm.</td>
</tr>
<tr>
<td>Thickness of anterior lamina</td>
<td>1 mm.</td>
<td>1-5 mm.</td>
</tr>
<tr>
<td>Marrow cavity</td>
<td>3 mm.</td>
<td>5 mm.</td>
</tr>
<tr>
<td>Thickness of posterior lamina</td>
<td>0-5 mm.</td>
<td>1 mm.</td>
</tr>
<tr>
<td>Thickness of skin and subcutaneous tissue over sternum</td>
<td></td>
<td>average 3 mm.</td>
</tr>
</tbody>
</table>

Thus a distance of 0-5-0-8 cm. is the average through which the needle has to pass in order to enter the marrow space.

The operation

The needle used in the cases recorded here was the one devised by Dr. Salah of the Research Institute of Cairo, and consists of an ordinary lumbar puncture needle cut short and fitted with a movable shield which can be fixed at any required distance to limit the depth of penetration (fig. 3).

The skin is first cleaned and disinfected with tincture of iodine, and some novocain is injected subcutaneously over the middle of the sternum opposite the second interspace. The shield is fitted at 0-5-0-8 cm. from the point of the needle, according to the age of the patient, the thickness of the sternum and the amount of the subcutaneous fat. The marrow cavity opposite the second intercostal space is readily located between the second and third ribs just below the prominent sterno-manubrial ridge. The needle with the stilette in place is inserted carefully. Because the suction necessary for aspirating the marrow must be fairly strong, a 10-c.c. syringe is used, even though a small amount of marrow fluid (from 0-1 to 0-2 c.c.) is all that is needed for morphological study. Aspiration of larger quantities in infants and children is inadvisable, since the marrow cavities are too small to permit the removal of material in large quantities. Besides, aspiration of large amounts of marrow would mean admixture...
with it of the peripheral blood circulating in the medullary sinuses. In fact, when as much as 2 c.c. of material are removed, they consist almost exclusively of blood, and even when a few drops only are aspirated the material obtained consists of bone marrow mixed with blood.

The only time the patient experiences any pain or discomfort is during the aspiration of the marrow, and the larger the quantity aspirated the more marked is the pain, which is often described as a sensation of suction. This pain is a sure sign that the needle is right in.

A collodion dressing is applied to the site of the puncture, which heals quickly. Films are prepared, dried quickly and stained by Giemsa or May-Grunwald-Giemsa method. A total nucleated cell count can also be determined. At least 500 cells are counted and recorded in terms of percentage of the total number of the nucleated elements including the cells of the erythrogenic series.

Marrow of normal subjects

The criteria of normal bone marrow in infants and children have not yet been adequately determined. The principal published works are those of Kato (1937), Vogel and Bassen (1939) and of Tecilazic (1935). Kato’s observations on fifty-one normal infants and children between the ages of one month and thirteen years revealed certain differences from the sternal marrow in adults. These differences are: (1) the relatively high percentage of the erythrogenic and lymphoid elements; (2) the relatively low percentage of the myeloid elements due to a lack of more mature granulocytes; the myelocytes and to a lesser degree the metamyelocytes forming the highest percentage of the myeloid elements, whereas the fully mature segmented forms are relatively few in number, being present to the extent of less than 7 per cent. This reversal of the percentage in erythrogenic and myeloid elements explained the low myeloid-erythroid ratio of 1 to 2 for infants and young children below four years, older subjects having a value of 3 to 4. The nucleated red cells ranged between 18 and 26 per cent.

Vogel and Bassen reported their observations on forty-one children. The rate of the myeloid to the erythroid cells was approximately 3 to 1. The myeloid cells showed a marked variation in different cases and the segmented neutrophils showed a higher percentage than in Kato’s series, 6 to 34 per cent. as compared with 4 to 7 per cent. Tecilazic, working with tibial marrow of normal new-born children, found a marked predominance of the erythroid elements (70 per cent.) in the early days of life. This excess is apt to diminish during the second week of life to about 45 per cent. From then on the tendency is towards diminution of the erythroid elements until about the age of two years, when the figures are likely to approach those for adults. In Kato’s series the erythroid elements ranged between 18 and 26 per cent., and showed practically no variations in the different age groups.

In the present series the number of normal cases examined was small. Fifteen cases in all, five infants from one year three months to two years old, and ten children from three years six months to five years five months old have been investigated. The result of the differential and total counts are detailed in tables 1 and 2. Preliminary blood and clinical examinations were relied upon to exclude any pathological conditions that might influence the marrow
Table 1
DETAILS OF THE TOTAL AND DIFFERENTIAL COUNTS OF MARROW SMEARS FROM TEN NORMAL CHILDREN

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE</th>
<th>SEX</th>
<th>TOTAL COUNT</th>
<th>MYELOBLAST</th>
<th>NEUTROPHIL</th>
<th>EOSINOPHIL</th>
<th>BASOPHIL</th>
<th>MONOCYTES</th>
<th>LYMPHOCYTES</th>
<th>ERYTHROBLASTS</th>
<th>NORMOBLASTS</th>
<th>MYELOID ERYTHROID RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. K. R. O.</td>
<td>4 7</td>
<td>Male</td>
<td>96,000</td>
<td>0.75</td>
<td>0.5 16.25 20-5 13 8</td>
<td>7.5 2 - 0.5</td>
<td>-</td>
<td>15</td>
<td>8</td>
<td>8</td>
<td>4.25</td>
<td></td>
</tr>
<tr>
<td>2. R. A. R.</td>
<td>4 4</td>
<td>Male</td>
<td>82,000</td>
<td>2</td>
<td>13 25 28 4 7.5 2-25</td>
<td>2 - 0.75</td>
<td>-</td>
<td>12</td>
<td>13.5</td>
<td>5</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3. M. S.</td>
<td>5 5</td>
<td>Male</td>
<td>76,000</td>
<td>0.25</td>
<td>- 11 24.5 10 16</td>
<td>2-5 2 - 4</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>6</td>
<td>7</td>
<td>5.5</td>
</tr>
<tr>
<td>4. M. S.</td>
<td>4 4</td>
<td>Male</td>
<td>140,000</td>
<td>0.25</td>
<td>- 13 22 13 17 4</td>
<td>- - 2.5</td>
<td>- 0.4</td>
<td>20</td>
<td>3-75</td>
<td>2.5</td>
<td>6-75</td>
<td></td>
</tr>
<tr>
<td>5. A. G.</td>
<td>5 5</td>
<td>Male</td>
<td>120,000</td>
<td>2</td>
<td>0.25 13 16 9 15</td>
<td>1-5 2-5 - 9</td>
<td>-</td>
<td>-</td>
<td>20-5</td>
<td>9</td>
<td>2-5</td>
<td>5-5</td>
</tr>
<tr>
<td>6. N. E.</td>
<td>4 4</td>
<td>Female</td>
<td>96,000</td>
<td>1.5</td>
<td>- 9 22 14 10 5</td>
<td>0.5 - 2.5</td>
<td>- 3</td>
<td>22.5</td>
<td>7.5</td>
<td>2.5</td>
<td>6.75</td>
<td></td>
</tr>
<tr>
<td>7. F. A.</td>
<td>4 3</td>
<td>Male</td>
<td>75,000</td>
<td>1</td>
<td>0.5 26 24 19 4 2 0.25 0.25 0.75</td>
<td>- 0.75</td>
<td>-</td>
<td>16.5</td>
<td>3</td>
<td>2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>8. A. R.</td>
<td>4 4</td>
<td>Male</td>
<td>160,000</td>
<td>0.75</td>
<td>0.25 7.5 17 16 12.5 5 3</td>
<td>- 1.25</td>
<td>-</td>
<td>22.25</td>
<td>11</td>
<td>3-5</td>
<td>4-2</td>
<td></td>
</tr>
<tr>
<td>9. S. A.</td>
<td>3 6</td>
<td>Male</td>
<td>150,000</td>
<td>0.75</td>
<td>1.25 26 13.5 12 7 1.5</td>
<td>- - - 1</td>
<td>-</td>
<td>27</td>
<td>5-5</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>10. M. A.</td>
<td>4 4</td>
<td>Male</td>
<td>120,000</td>
<td>0.50</td>
<td>2 24 15.5 10 8</td>
<td>2.5 - - 1.5</td>
<td>-</td>
<td>27</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
DETAILS OF THE NUCLEATED AND DIFFERENTIAL COUNTS OF MARROW SMEARS FROM FIVE NORMAL INFANTS

<table>
<thead>
<tr>
<th>CASE</th>
<th>AGE</th>
<th>SEX</th>
<th>TOTAL COUNT</th>
<th>MYELOBLAST</th>
<th>NEUTROPHIL</th>
<th>EOSINOPHIL</th>
<th>BASOPHIL</th>
<th>MONOCYTES</th>
<th>LYMPHOCYTES</th>
<th>ERYTHROBLASTS</th>
<th>NORMOBLASTS</th>
<th>MYELOID ERYTHROID RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. M. G.</td>
<td>10</td>
<td>Male</td>
<td>280,000</td>
<td>1-25</td>
<td>0.5 23 15 3 8.75</td>
<td>1.5 0.25 -</td>
<td>-</td>
<td>37.5</td>
<td>4-25</td>
<td>5.5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2. S. A.</td>
<td>1 3</td>
<td>Male</td>
<td>700,000</td>
<td>2</td>
<td>0.25 16 11.5 8.5 1.5</td>
<td>8 0.75 - 1.25</td>
<td>-</td>
<td>31.25</td>
<td>16.25</td>
<td>9</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>3. N. G.</td>
<td>2 2</td>
<td>Female</td>
<td>500,000</td>
<td>1-25</td>
<td>1-25 23.75 21.5 6.5 2.75</td>
<td>2.5 0.25 - 1</td>
<td>0.25 22.75</td>
<td>12.75</td>
<td>5.5</td>
<td>3.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Y. F.</td>
<td>10</td>
<td>Male</td>
<td>309,000</td>
<td>1</td>
<td>2 15 19 6.5 13.5</td>
<td>4 2 - -</td>
<td>-</td>
<td>13-5</td>
<td>15</td>
<td>7.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>5. R. M.</td>
<td>2 3</td>
<td>Male</td>
<td>300,000</td>
<td>2-5</td>
<td>0.25 7.5 16 7.5 8.5</td>
<td>1 0.5 - -</td>
<td>-</td>
<td>33.5</td>
<td>16.5</td>
<td>6.5</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>
picture. In the children of the series the findings could be summarized as follows:

1. The total nucleated cell count varied from 75,000 to 160,000 per c.mm.
2. There was a definite predominance of the myeloid elements. The myeloid-erythroid ratio was 4 to 6, and in two cases it was 9 and 13 respectively (cases 4 and 7). The percentage of the neutrophil segmented forms was generally low. In six out of the ten cases it was below 10, and in the rest of the cases it was about 15. The myelocytes, metamyelocytes and to a lesser degree the staff forms form the greatest percentage of the myeloid elements. In Kato's series the fully mature segmented forms were few in number, being less than 7 per cent.
3. The percentage of eosinophils was high. In five cases it ranged between 4 and 9, and in two cases it was 10 and 13 respectively.
4. The relatively high percentage of lymphocytes in most of the cases was striking.
5. The cells of the red series display a great variation from 5 to 18·5 per cent. In most of the cases the readings were lower than those of Kato in the same age groups (18 to 25 per cent.).

The majority of the cells were erythroblasts. In Kato's series the cells were mostly normoblasts. Megablasts were absent in all the cases. Early erythroblasts in mitosis were frequently seen.
6. The myeloblasts averaged only from 0·25 to 2 per cent.
7. Megakaryocytes were invariably present.

In the infants of the series (between one and two years) the following differences from the older children were noted: (1) the total nucleated count was definitely higher, varying from 300,000 to 700,000 per c.mm.; (2) the percentage of lymphocytes was generally higher: in three cases it was above 30 and in one case it reached up to 37·5 per cent.; (3) the percentage of neutrophil segmented forms reached a lower level: in four out of five cases it was below 10 and in one case it was only 1·5 per cent. (table 2).

**Bone marrow in certain pathological conditions**

**Ankylostoma anaemia.** The examination of the bone marrow of ten cases of ankylostoma anaemia revealed definite changes in its morbid anatomy. In nine out of the ten cases the marrow showed a marked degree of hyperplasia which was essentially erythroblastic and normoblastic. The total nucleated cell count in the marrow was generally between 250,000 and 300,000 per c.mm., but in two cases it was 1,120,000 and 2,790,000 respectively, thus indicating intense hyperplasia. The cytoplasm of the nucleated red cells was commonly basophilic or polychromatic, and numerous mitotic figures were seen in almost every case. Eosinophils were invariably present (fig. 7). This picture indicates an intense bone marrow stimulation similar to that obtained in post-haemorrhagic anaemias and favours the view that ankylostoma anaemia is due to chronic
bleeding. In one case the eosinophilic reaction was striking (22.2 per cent.) and was the main feature, the erythro-normoblastic reaction being slight.

Fig. 4.—A microphotograph of the marrow of a case of ankylostoma anaemia. Note increase in the number of erythroblasts and normoblasts.

The findings of the sternal puncture may indicate a strong suspicion of ankylostoma infestation, though stools may at first be reported negative and repeated examination may ultimately demonstrate ankylostoma ova, as the following two cases show:

Case 1. A. M., a boy six years old, was admitted for pellagra and severe anaemia. First examination of the stools revealed no ankylostoma ova. As sternal puncture showed an erythro-normoblastic reaction (37 per cent.), stools were re-examined and on the third occasion ankylostoma ova were found.

Case 2. S. M. E., a girl eleven years old, in whom the first examination of the stools revealed only ascaris ova, had on sternal puncture a marked erythro-normoblastic reaction (43 per cent.). The stools were re-examined and ankylostoma ova were detected at the fourth attempt.

EFFECT OF IRON THERAPY. The administration of iron produces a gradual diminution of the general cellularity, and at the same time maturation is stimulated. The nucleated red cells gradually return to their normal level, and
when iron therapy is completed, the bone marrow resumes its normal character, as exemplified by the record of the following case:

Case 3. A. M. E., a boy of twelve years of age, was admitted for pellagra, progressive wasting and pallor. Examination of the stools revealed ankylostoma ova. Blood examination showed 30 per cent. haemoglobin, red blood cells 2,634,000 per c.mm. and eosinophils 10 per cent. Sternal puncture revealed a definite erythro-normoblastic reaction (40-5 per cent.). Carbon tetrachloride was given as an anthelminthic. Iron was given for a month, after which another sternal puncture was done. It revealed a diminution of the general cellularity, and the erythroblasts and the normoblasts approached the normal figure (22-5), and the myeloid-erythroid ratio rose from 1-5 to 4-3.

The presence of a mixed ascaris and ankylostoma infestation does not seem to alter the characteristic bone marrow picture of ankylostoma anaemia. The marrow of cases with pure ascaris infestation was examined and no characteristic picture was revealed.

BILHARZIASIS. The bone marrow of five cases of urinary bilharziasis was examined. Four cases showed a moderate eosinophilic reaction ranging between 10 and 15. The fifth case, that of a child ten years old, revealed a high eosinophilia in the differential count of the peripheral blood (70 per cent.) and a study of the bone marrow showed an intense eosinophilic reaction of 60 per cent. (fig. 5).

CRETINISM. The sternal marrow of two cases of cretinism was examined. The main feature in the two cases was the low total count, indicating hypoplasia of the bone marrow. This depression is most probably the result of the general slowing down of metabolic and other processes of the body in hypo-thyroid states.
In one case, aged twelve years, the total nucleated cell count was 40,000 per c.mm., and rose after one month's thyroid treatment to 190,400. The differential count was as follows: neutrophil myelocytes 16, metamyelocytes 11, staff cells 18, segmented 20, eosinophils 2·4, monocytes 1·4, myeloblasts 0·4, lymphocytes 20·4, erythroblasts 6, normoblasts 5·2 per cent. The effect of thyroid on the general cellularity of the marrow was striking (fig. 6 and 7), and the nucleated red cells gave a higher figure of 15 per cent. The haemoglobin in the peripheral blood rose at the same time from 30 to 60 per cent. at the end of the treatment.

In the second case, aged three years, the total nucleated cell count was 67,700 per c.mm. The differential count was as follows: myelocytes 18, metamyelocytes 20, staff cells 12, segmented 12, eosinophil 5·2, monocytes 0·4, lymphocytes 11, erythroblasts 13, normoblasts 8, punctate basophilia 0·4 per cent., myeloid-erythroid ratio 3·1.
Summary

1. A radiological and naked eye study of the human sternum was carried out to determine the developmental stages of the ossification centres and to know the correct depth to which the needle should be plunged in order to reach the marrow cavity.
2. The details of the technique of sternal puncture are discussed.
3. A study of the marrow of ten normal children from three years six months to five years five months old and five infants from one to two years old has been carried out.
4. The bone marrow picture in ankylostoma anaemia has been studied. An erythro-normoblastic reaction is the outstanding feature.
5. The results of examination of the marrow of two cases of cretinism and five cases of bilharziasis are reported.
References