An Evaluation of Paraffin Sections of Aspirated Bone Marrow in Malignant Lymphomas

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STUDIES of the peripheral blood and aspirated bone marrow in malignant lymphomas have for the most part proved disappointing as far as demonstration of specifically diagnostic features is concerned. Wintrobe stated that the chief value of bone-marrow study in these conditions is to rule out leukemia, especially the aleukemic form. In an attempt to evaluate the clinical usefulness of bone-marrow aspiration as a means of diagnosis in malignant lymphomas, one of us (Cooper) and Watkins reviewed the material available at the Mayo Clinic prior to 1949. It was found that marrow aspiration was of no help in cases of Hodgkin’s disease or follicular lymphoma but that it was of value in some cases of lymphosarcoma.

Since this article was written, the examination of material obtained by aspiration of bone marrow has included the study of paraffin serial sections of marrow fragments. The present study is undertaken in order to evaluate this additional procedure in the diagnosis of malignant lymphomas.

The literature with regard to results of sternal aspiration in malignant lymphomas has been reviewed by Cooper and Watkins, Limarzi and Paul, and one of us (Pease) and will not be reviewed here. Very few observers have found the procedure to be of value in these conditions, although necropsy examinations indicate marrow involvement in a high percentage of cases. Significantly, perhaps, most reports have not included the examination of paraffin sections of the aspirated marrow.

Material and Methods

In preparation for this study all records of bone-marrow aspiration obtained during the period from January 1, 1950, through August 31, 1953, were reviewed, and all cases in which the diagnosis of malignant lymphoma was established were selected for further study, provided examination of the peripheral blood did not suggest leukemia. Seventy-two cases were found to meet these criteria. In 65 cases the diagnosis was confirmed by biopsy of other tissue or by necropsy. In seven cases physical findings and the subsequent clinical course of the patient were compatible with the diagnosis. Paraffin sections of aspirated bone marrow and histories of these cases were reviewed. An attempt was made to evaluate the extent of the disease at the time of marrow aspiration and to correlate this with laboratory findings. For purposes of comparison, 48 cases in which benign aggregations of lymphocytes had been noted on examination of paraffin sections were also reviewed.

Technic of Examination of Aspirated Marrow

Approximately 2 ml. of sternal bone marrow were aspirated and placed in a paraffin-lined vial to which dog-liver heparin had been added to prevent clotting. Dry films of this material were stained with Wright’s stain and the marrow particles or “units” were made into

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paraffin sections after fixation with 4 per cent formalin in saline solution. Serial sections were made from the paraffin blocks. Every other slide was stained with hematoxylin and eosin. Alternate slides were saved for special stains if indicated.

RESULTS OF STUDY

Hodgkin's Disease

There were 34 cases of Hodgkin's disease. In nine cases paraffin sections of aspirated marrow revealed lesions which were diagnostic. The lesions were classified as Hodgkin's disease on the basis of replacement of marrow by sheets of tissue of a pleomorphic character. Large eosinophilic reticulum cells predominated, but these were associated with fibroblasts, eosinophils, lymphocytes and the characteristic Reed-Sternberg cells (fig. 1a and b). In four cases the involvement was diffuse, with lymphomatous tissue infiltrating almost all units. In the remaining five cases, however, only a few units were involved and the remaining marrow appeared normal (fig. 1a). Serial sections are most useful in such cases, since by examining only random sections one might easily miss the discrete lesion.

In two of the cases, Reed-Sternberg cells were seen in smears of the aspirated marrow* but in the remaining seven cases smears were not helpful.

In three cases the diagnosis was based on marrow findings alone. Two patients have died and in one of them a necropsy was performed which confirmed the diagnosis. In the third case, a review of splenic tissue removed some months prior to marrow aspiration revealed lesions of Hodgkin's disease. In the remaining cases diagnosis had been made on the basis of tissue biopsy prior to examination of the bone marrow.

In five cases small nonspecific granulomatous lesions were noted. These consisted of a central core of swollen reticulum cells, which had the appearance of epithelioid cells, surrounded by lymphocytes and eosinophils and occasionally fibroblasts (fig. 1c). No observation was made of giant cells or central necrosis. This type of lesion has been reported previously in patients with malignant lymphoma, but is also seen in many other diseases, such as tuberculosis, sarcoidosis and infectious mononucleosis. In a study of 50 cases in which granu-

* These cases have previously been reported by Bayrd and associates.2
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lomatous lesions were found in the marrow, one of us (Pease) found that nine of the patients had a diagnosis of lymphoma made on the basis of biopsy of other tissue. The lesion differed in no way from that found in other conditions, and can only be considered as a nonspecific though pathologic finding.

Although there were exceptions, in general those patients who had lymphomatous or granulomatous lesions in their marrow were found clinically to have more advanced disease than those in whom marrow aspiration was negative. There was no significant difference in the length of time they had had symptoms, however.

Only one patient of the nine in whom lesions were found in the marrow had normal peripheral blood. The remaining patients had either a severe anemia or a marked leukopenia and thrombocytopenia. Of the 20 patients whose marrow aspirations were not diagnostic, 10 had either a severe anemia, leukopenia or thrombocytopenia. Six of these patients had palpable enlargement of the spleen, and it is possible that “hypersplenism” might have accounted for the peripheral blood changes. No explanation is apparent in the remaining four cases. It is quite possible that a single marrow aspiration failed to remove lymphomatous tissue from a focally involved organ, giving a false negative result.

Comment. The much higher incidence of positive results obtained in these cases than in those reported by Cooper and Watkins indicates the importance of examination of serial paraffin sections of bone marrow in cases of this type. In three cases the diagnosis would not have been made without marrow examination. The presence of a severe abnormality of the peripheral blood in patients suspected of having this disease should lead one to expect marrow involvement, and it is possible that repeated examination might be helpful in case the first aspiration were negative.

Lymphosarcoma, Reticulum Cell Type

There were 11 cases of reticulum cell sarcoma. In six cases a diagnosis of malignant lymphoma could be made on paraffin sections of marrow units. In four of these the diagnosis was based on the replacement of normal marrow by sheets of large eosinophilic cells with indefinite cell borders, large nuclei, prominent nucleoli and numerous mitotic figures (fig. 2). Pleomorphism was not apparent, as it was in cases of Hodgkin’s disease, and no Reed-Sternberg cells were seen. In two of these cases involvement was diffuse, while in two the involvement was focal and lesions could easily have been missed if random sections alone had been examined. In one case, in addition to aggregations of large eosinophilic reticular cells, these cells were diffusely interspersed throughout the marrow as well. In the final case there was a marked diffuse infiltration of the marrow by both small lymphocytes and large eosinophilic reticular cells.

In only three cases were the findings on smears of the marrow abnormal. In two the predominant cell was a large, bizarre, reticular cell and in one a mass of immature lymphocytes was seen. In the two former cases a definite diagnosis

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**Fig. 1.** Aspirated marrow from patients with Hodgkin’s disease. a. One unit is completely replaced by large eosinophilic reticulum cells, lymphocytes and Reed-Sternberg cells. b. High-power view to show many Reed-Sternberg cells. c. Small collection of reticulum cells resembling a granuloma. Similar lesions are found in other types of lymphoma as well as in other unrelated conditions.
could not be made on the basis of smear alone and there was inadequate material for diagnosis of the paraffin sections on the first aspiration. In each case a repeated marrow aspiration produced sufficient material for a definite diagnosis by paraffin sections. In the remaining cases marrow smears were not helpful.

In one case granulomatous lesions were found. These were similar to those seen in the patients with Hodgkin's disease, and were again considered pathologic but nonspecific.

In general the patients in this group who were found to have lymphomatous lesions in the marrow tended to have more advanced disease than those with nondiagnostic marrow, but the difference was less marked than in patients with Hodgkin's disease. Abnormalities of the peripheral blood were also not as marked nor as frequent, and the difference between those with diagnostic and those with nondiagnostic marrow was not significant.

Comment. The importance of repeated marrow aspiration when material is suggestive but not diagnostic is indicated in this group of cases. In two cases a definite diagnosis could not have been made if this had not been done. Here again, as in Hodgkin's disease, examination of serial sections of marrow units proved to be more helpful than examination of smears.

**Lymphosarcoma, Lymphocytic Type**

There were 21 cases of lymphocytic lymphosarcoma. In six of these, lesions compatible with malignant lymphoma were observed on examination of paraffin sections of bone marrow, and in five the findings were considered to be suggestive although not diagnostic. In the remaining 10 cases sections were neither suggestive nor diagnostic.

It was in this group that the greatest difficulty in interpretation of the paraffin sections was encountered. Benign aggregations of lymphocytes not infrequently occur in the marrow, and a sharp differentiation between these benign aggre-
aggregations and those which represent malignant lesions is not always easy to make. For this reason a group of 48 cases in which benign aggregations of lymphocytes had been noted were selected for study and comparison. The results of this comparison will be discussed later in this paper.

Of the cases in which paraffin sections were considered diagnostic, two patients had such diffuse replacement of the marrow by lymphocytes as to leave little doubt of the malignancy of the process (fig. 3a). Many of the cells had large nucleoli, and mitotic figures were fairly numerous. Three patients had numerous large aggregations of lymphocytes which were not well demarcated from sur-

Fig. 3.—Aspirated marrow from patients with lymphosarcoma, lymphoeytic type. a. Many marrow fragments are completely replaced by small lymphocytes. Very little normal marrow was found in this case. b. Involvement of a large area by lymphocytes, with poor demarcation from surrounding marrow tissue.
rounding marrow tissue and seemed to be invading large areas (fig. 3b). The cells in these aggregations tended to be somewhat larger than normal lymphocytes, and nucleoli were prominent. No “reaction centers” were seen, and in some cases immature lymphocytes were seen near the periphery of the aggregations. There was no evidence of organization into a lymphoid follicle. In one case there were both diffuse and focal infiltrations by large eosinophilic cells similar to those seen in the reticulum cell sarcoma group. This would have been the diagnosis made on marrow examination, but a lymph node removed subsequently revealed the small round cell type of lymphocytic lymphosarcoma.

In five cases where the sections were considered to be suggestive only, changes similar to those described in the preceding paragraph were observed, but they were not as marked. In three cases there was diffuse infiltration by lymphocytes, but these cells were not as numerous and the malignant characteristics were not sufficient to make a definite diagnosis of malignant lymphoma. The normal marrow pattern seemed undisturbed by the infiltrating lymphocytes. In two cases there were lymphocytic aggregations, but again these were not numerous and their malignancy was not as definite as in the previous group.

In all 11 of the cases in which paraffin sections were considered either diagnostic or suggestive of lymphosarcoma, smears of the aspirated bone marrow were very helpful. In each case there was an increase in immature lymphocytes noted, and smears were considered to be suggestive of lymphosarcoma in eight cases and diagnostic in three. A combination of smear and paraffin-section examination made the diagnosis possible in each case.

In six cases the final diagnosis was based on the findings of marrow aspiration alone. In three of these, examination of other tissue subsequently confirmed the diagnosis. In the remaining three, physical findings and the clinical course were compatible with this diagnosis.

In one case in this group no material entered the syringe during aspiration. There was a trace of material in the needle, however, and a smear was made of this material. The needle was then rinsed with saline solution and the tiny bits of tissue which it contained were made into paraffin sections. Examination of both specimens revealed material diagnostic of malignant lymphoma.

Abnormalities of the peripheral blood were not as marked in these cases as in those patients with Hodgkin’s disease or reticulum cell sarcoma. Platelet counts were decreased in all cases in which they were done and six patients had a moderate increase in either lymphocytes or monocytes, but only one patient had a severe anemia, which was associated with leukopenia. Interestingly enough, this patient did not have diagnostic findings on marrow aspiration.

There was no marked difference in the clinical estimate of the extent of the disease in those patients with and without diagnostic lesions in the marrow.

Comment. Difficulties in diagnosis of paraffin sections were greatest in this group, and smears of marrow aspiration were more helpful than in the other types of lymphoma. Benign aggregations of lymphocytes are not infrequently seen in otherwise normal marrow, and it is not always easy to differentiate the aggregations from those which represent malignant lymphoma.

One case in this group emphasizes the importance of examining all material even when there is an apparent “dry tap.” In the hands of an experienced oper-
ator, the occurrence of a dry tap should arouse suspicion of gross abnormality of the marrow, and every attempt should be made to examine even the smallest amount of tissue.

Follicular Lymphoma

There were six cases of follicular lymphoma included in this study. In one case there was a marked diffuse increase of lymphocytes seen in paraffin sections of bone marrow and a diagnosis of malignant lymphoma was made. In two additional cases lesions which were considered suggestive were seen. In one of these the infiltration by lymphocytes was diffuse and in the other there were a few aggregations of lymphocytes which, although they lacked the appearance of complete benignancy, did not exhibit definite malignant characteristics. In all three cases lymph-node biopsy done within a few days of the marrow examination was diagnostic.

Smears of the marrow in these cases were not helpful. In three cases some increase in lymphocytes was noted, but in no case was a diagnosis of malignant lymphoma suggested.

Abnormalities of the peripheral blood were also not marked, although thrombocytopenia was noted in three cases and severe anemia in one of these. Granulomatous lesions were found in one patient from this group. The clinical extent of the disease seemed to be less advanced in those patients with marrow lesions than in those without, although the group is too small to permit any conclusions.

Comment. Although only two of the six patients in this group had completely negative marrow examination, the procedure was less helpful in this type of lymphoma than in any other type. In only one case were paraffin sections diagnostic of malignant lymphoma, and even here no classification of the type of lymphoma was possible. In each case other material was available for diagnosis.

Benign Lymphocytic Aggregations

In 13 of the patients with malignant lymphoma, aggregations of lymphocytes were noted but were considered to be benign. A review of approximately 1,750 marrow examinations of patients with miscellaneous disorders revealed that similar aggregations had been sufficiently prominent or numerous to be mentioned in 48 cases. The sections were studied from the standpoint of cellularity, number and size of lymphocytic aggregations, the presence or absence of a central vessel, a reaction center and surrounding eosinophilia, the degree of circumscription and the morphology of the cells. In none of these factors did the 13 cases of malignant lymphoma in which incidental lymphocytic aggregations were found differ from the benign group, and it is assumed that in these cases the lymphocytic aggregations were of no significance.

The factors which were most significant in distinguishing between benign and malignant aggregations of lymphocytes were the number of these aggregations, the cellular morphology, and to some extent the degree of circumscription and the presence or absence of “reaction centers.” Benign aggregations were fewer, tended to be more circumscribed, occasionally contained reaction centers, often surrounded a small vessel, and were made up of small lymphocytes of benign
FIG. 4.—Aspirated marrow from a patient with periarteritis nodosa. A fairly well circumscribed collection of lymphocytes with no cytologic evidence of malignancy. Similar aggregations are found not infrequently in a large number of unrelated conditions.

appearance (fig. 4). In the three cases in which lymphocytic aggregations were considered malignant, the aggregations were more numerous and somewhat less circumscribed than in benign lesions, and the individual cells were larger and nucleoli and mitotic figures were more frequent. The aggregations which were considered suggestive of lymphoma were considered so on the basis of the morphologic characteristics of the cells. The diagnosis could not be made more definite because these changes were not sufficiently marked.

SUMMARY AND CONCLUSIONS

Examination of serial paraffin sections of bone-marrow aspirations has increased the known incidence of marrow involvement in malignant lymphomas. The results of this study can be seen in the table. In the 72 cases reviewed here, lesions compatible with malignant lymphoma were seen in paraffin sections in 22 cases. In 12 cases this was the only material available for diagnosis at the time. In an additional seven cases paraffin sections were considered suggestive of malignant lymphoma and in five of these a diagnosis could be made on the basis of examination of both smear and paraffin section. This procedure is particularly helpful in Hodgkin’s disease and reticulum cell sarcoma, where marrow smears are only rarely diagnostic.

In general, the patients in whom lesions were found in the bone marrow tended to have more advanced disease than those in whom lesions were not found, and abnormalities in the peripheral blood were more common. This was especially true of patients with Hodgkin’s disease, less so in those with reticulum cell sarcoma. Patients with lymphosarcoma, lymphocytic type, did not show this difference, either in the extent of the disease or in findings on examination of peripheral blood.
Diagnosis was most difficult in patients who had lymphocytic lymphosarcoma, since it was occasionally difficult to distinguish between the benign aggregations of lymphocytes which are not infrequently found in a large number of unrelated conditions and those aggregations which represented malignancy. Differentiation was made on the basis of number of aggregations, degree of circumscription, presence or absence of reaction centers and the cytologic appearance of the cells. Examination of the smears of the marrow is most helpful in these cases.

Seven patients in this series (9.7 per cent) had granulomatous lesions in the marrow. In five of these the final diagnosis was Hodgkin’s disease, in one reticulum cell sarcoma and in one follicular lymphoma. Although these lesions were not specific, the incidence is high enough to make the finding of a granuloma of some significance in any patient suspected of having malignant lymphoma.

The importance of thorough examination of any material obtained in an apparent dry tap and the value of repeated marrow examinations is emphasized by three cases in this series.

Since in many cases diagnostic lesions are found in only one portion of the material, examination of only one section of the paraffin sections of aspirated bone marrow is not sufficient for proper evaluation. This fact is emphasized by the greater incidence of positive findings in this report than in that of Cooper and Watkins.

Studies of bone marrow should be particularly helpful in instances in which the diagnosis of malignant lymphoma is suggested by clinical features but cannot be proved by biopsy of peripheral nodes or other readily accessible tissue.

SUMMARIO E CONCLUSIONES IN INTERLINGUA

Nostre examine de series de sectiomies a paraffin de aspirate medulla ossee ha revelate un frequentia plus alte que previemente reportate de involvimento del medulla in casos de maligne lymphoma. Inter le 72 casos hie revisite, 22 monstrava in sectiones a paraffin de medulla signos de lesiones compatibile con lymphoma maligne. In 12 del casos isto esseva le sol material diagnostic disponibile al tempore del examine. In septe casos additional le sectiones a paraffin suggereva al minus le conjectura de lymphoma maligne, e in cinque de illos le examine supplementari de frottis permitteva un diagnose definitive. Le examine de sectiones a paraffin es specialmente utile in casos de morbo de Hodgkin e de sarcoma de cellulas del texito reticular ubi frottis medullar es rarmente diagnostichamente inequivoc.

In general, le patientes in qui lesiones esseva trovate in le medulla ossee tendeva a haber lor morbo in stadios plus avanitate que illes in qui nulle lesions esseva trovate. Le patientes con lesiones medullar esseva etiam caracterisate per un plus alte frequentia de anormalitates del sanguine peripher. Iste differentias esseva specialmente pronunciate in casos de morbo de Hodgkin; illos esseva minus clar in casos de sarcoma del texito reticular. In patientes con lymphosarcoma (del typo lymphocytic) nulle tal differentia esseva constatate.

Le diagnose esseva specialmente difficile in patientes con lymphosarcoma lymphocytic. Le ration esseva que in alineas de iste casos il non esseva facile distinguir inter le benigne aggregaciones de lymphocytos (que se incontra non infrequentemente in un grande numero de conditiones non affin) e le aggrega-
tiones de character maligne. Le differentiation esseva establit super le base del numero de aggregationes, le grado de circumscription, le presentia o absentia de centros de reaction e le apparentia cytologie del cellulas. In tal casos le examine de frottis medullar es utilissime.

Septe patientes in le integre serie (i.e., 9,7 pro cento) habeva lesions granulomatose in le medulla. Pro cinque inter illes le diagnose final esseva morbo de Hodgkin; pro un, sarcoma de cellulas del texito reticular; e in un, lymphoma follicular. Ben que iste lesions non esseva specific, lor frequentia esseva sufficientemente alte pro que illos sia considerate como digne de attention in omne patientes sub suspicion de lymphoma maligne.

Tres casos del serie illustra le importantia de repetite examines del medulla e de un exacte studio de omne tracía de material trovate in le agulia quando le aspiration lassa le syringa completemente vacue.

Proque in multe casos lesions diagnostic se trova solmente in un portion del material, le examine de solmente un section a paraffin de aspirate medulla ossee non suffice pro un adequate evalutation. Iste facto explica le plus alte frequentia de resultatos positive in iste reporto que in illo de Cooper e Watkins.

Studios del medulla ossee es specialmente utile in casos in que un diagnose de lymphoma maligne es sugerite per signos clinic sed non es demonstrabile super le base de biopsia de nodos peripheric o de altere texito que es facilmente accessibile.

REFERENCES

An Evaluation of Paraffin Sections of Aspirated Bone Marrow in Malignant Lymphomas

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