The Value of Routine Bone Marrow Culture for *Histoplasma Capsulatum* in Pediatric Hematology

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Schwartz and Barsky[1] recently reviewed their experiences with routine cultures of the bone marrow for *Histoplasma capsulatum*. Of their 193 cases, only 1 yielded a positive culture. They concluded that routine cultures of the bone marrow for *H. capsulatum* have little value in histoplasmosis case finding. We presume, since their cases were obtained from the medical and surgical wards of Cook County Hospital, that the majority of them were adults.

Serving an area that is heavily endemic for *H. capsulatum*[2] we have frequently been concerned with the diagnostic problem of histoplasmosis. Our experiences on the adult wards at the Indiana University Medical Center are similar to those of Schwartz and Barsky. On the pediatric wards of the James Whitcomb Riley Hospital for Children, however, the results of routine bone marrow culture stand out in sharp contrast.

Other authors [3–6] have reported on disseminated histoplasmosis in infants and children. Since none of these have contrasted their cases with the number of negative cultures obtained, and since we have found routine bone marrow culture for *H. capsulatum* to be of such value, it was thought that this report might be of some interest.

Case Material and Methods

Seventy-six patients who were admitted to the James Whitcomb Riley Hospital for Children as hematologic diagnostic problems were subjected to bone marrow culture. All culture specimens were obtained by bone marrow aspiration employing the technic which we have previously described.[7] After the specimens have been obtained for cytologic study, an additional 2 cc. are aspirated and ejected with sterile technic into a sterile test tube containing balanced oxalate. This fluid specimen is then taken immediately to the bacteriology laboratory where cultures for *H. capsulatum* are prepared. The oxalated bone marrow aspirate is planted on slants of Sabaraud's dextrose agar, and incubated at room temperature. If the specimen is adequate several cultures are made, and one of these is incubated at 37 C. to obtain a culture in the yeast phase. Cultures are examined grossly every two or three days. Usually growth has been observed at twelve to fourteen days at room temperature, but occasionally has not been seen until after three weeks or more of incubation. All cultures are kept a month before being considered negative.

Results

Of 76 patients studied, 5 yielded *H. capsulatum* on bone marrow culture. These cases with their associated findings are briefly summarized in table 1. Seventy-one proved negative (table 2). It should be emphasized that none of these 71 had a diagnosis of histoplasmosis established by other methods (lymph node biopsy,

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TABLE 1—*Positive Bone Marrow Cultures for* Histoplasma capsulatum

Case	Patient	Sex & Age	Bone Marrow Examination	Marrow Culture	Complement Fixation	Histoplasmin Skin Test	Course
1	D. B.	F. 17 mos.	*H. capsulatum* found in monocytes, plasma cells, & phagocytic clasmatocytes. Erythroid hyperplasia	Positive	2 plus in 1:4 dilution	Not Done	No response to ethyl vanillate therapy. Died November 2, 1951. Autopsy revealed disseminated histoplasmosis.
2	C. M.	F. 3 mos.	Diminution in lymphocytes. Increased no. of stimulated monocytes and epithelioid cells.	Positive	3 plus in 1:8 dilution	Positive 1:100 dilution	Excellent response to ethyl vanillate therapy. Living and asymptomatic when last seen on August 15, 1952, ten months after diagnosis was established.
3	L. S.	M. 4½ mos.	Pan Marrow Hyperplasia	Positive	Negative	Negative	Treated with ethyl vanillate. Downhill course. Expired June 25, 1952. Autopsy not obtained.
4	B. T.	F. 15 mos.	*H. capsulatum* found free in marrow and engulfed by phagocytic clasmatocytes, monocytes, and polymorphonuclear neutrophils.	Positive	Not Done	Negative	No response to ethyl vanillate therapy. Died June 16, 1952. Autopsy revealed disseminated histoplasmosis.
5	G. C.	M. 5½ yrs.	Pan Marrow Hyperplasia	Positive	Not Done	Not Done	Lost to clinic. Last known to be alive on July 19, 1952, two months after bone marrow culture was made.

abdominal surgical biopsy, sputum culture, or necropsy) after negative bone marrow cultures had been obtained.

Case Reports

Case 1 (D. B.)

This 17 month old white girl was admitted to the James Whitcomb Riley Hospital on October 18, 1951 because of anemia, fever and lethargy of one month's duration. Physical examination revealed a temperature of 101.5 F., shotty cervical lymph nodes and liver and spleen both palpable at the level of the umbilicus. Laboratory findings were as follows: hemoglobin, 8.7 Gm.; total erythrocyte count, 3.19 million. Total leukocyte count was 3050 with the following differential: 16 per cent polymorphonuclear neutrophils, 3 per cent metamyelocytes, 2 per cent basophils, 36 per cent small lymphocytes, 2 per cent intermediate lymphocytes, 4 per cent active monocytes, 20 per cent young monocytes and 17

Table 2—*Negative Bone Marrow Cultures for* Histoplasma capsulatum

1. Anemias	16 patients
2. Primary and Secondary Hypersplenic States	11 patients
3. Splenomegaly of unexplained etiology	9 patients
4. Collagen System Disease	7 patients
5. Acute Leukemias	5 patients
6. Acute Bacterial Infections	5 patients
7. Lymphadenopathy of unexplained etiology	4 patients
8. Non-thrombocytopenic purpuras	3 patients
9. Lipoid and Nonlipoid Reticuloendothelioses	2 patients
10. Miscellaneous	
a. Biliary Cirrhosis	2 patients
b. Leukopenia of unexplained etiology	1 patient
c. Eosinophilia of unexplained etiology	1 patient
d. Epistaxis of unexplained etiology	1 patient
e. Carcinoma	1 patient
f. Fever of unexplained etiology	1 patient
g. Myositis Ossificans	1 patient
h. Congenital Heart Disease	1 patient
Total	71 patients

per cent monoblasts. Total platelet count was 146,740. Bone marrow aspirate was hyperplastic. Granulocytes were left shifted to "B" myelocyte level. There was a considerable increase in monocytes with a fair number of monoblasts. *H. capsulatum* organisms were found free in the marrow and engulfed by phagocytic clasmatocytes, plasma cells, monocytes, and polymorphonuclear neutrophils (fig. 1). *H. capsulatum* also grew in culture of bone marrow. Complement fixation test for *H. capsulatum* was 2 plus in a 1:4 dilution. X-ray of the chest was interpreted as showing bilateral acute interstitial pneumonitis. The patient was placed on ethyl vanillate therapy. This drug was without effect, and patient expired on the fourth day of treatment. *H. capsulatum* was seen in large numbers in the peripheral blood smears on the day of demise. Autopsy revealed widespread lesions of histoplasmosis.

Case 2 (C. M.)

This 3 month old white female infant was admitted to the James Whitcomb Riley Hospital on October 21, 1951 because of cough, failure to gain weight and vomiting after feedings. Positive physical findings were limited to low-grade fever, splenomegaly and hepatomegaly. Hemogram revealed the following: hemoglobin, 8.7 Gm.; total erythrocyte count,

3.07 million. Total leukocyte count was 6650 with the following differential: 40 per cent polymorphonuclear neutrophils, 2 per cent metamyelocytes, 2 per cent "C" myelocytes, 1 per cent eosinophils, 1 per cent basophils, 36 per cent small lymphocytes, 5 per cent intermediate lymphocytes, 9 per cent active monocytes, 2 per cent young monocytes and

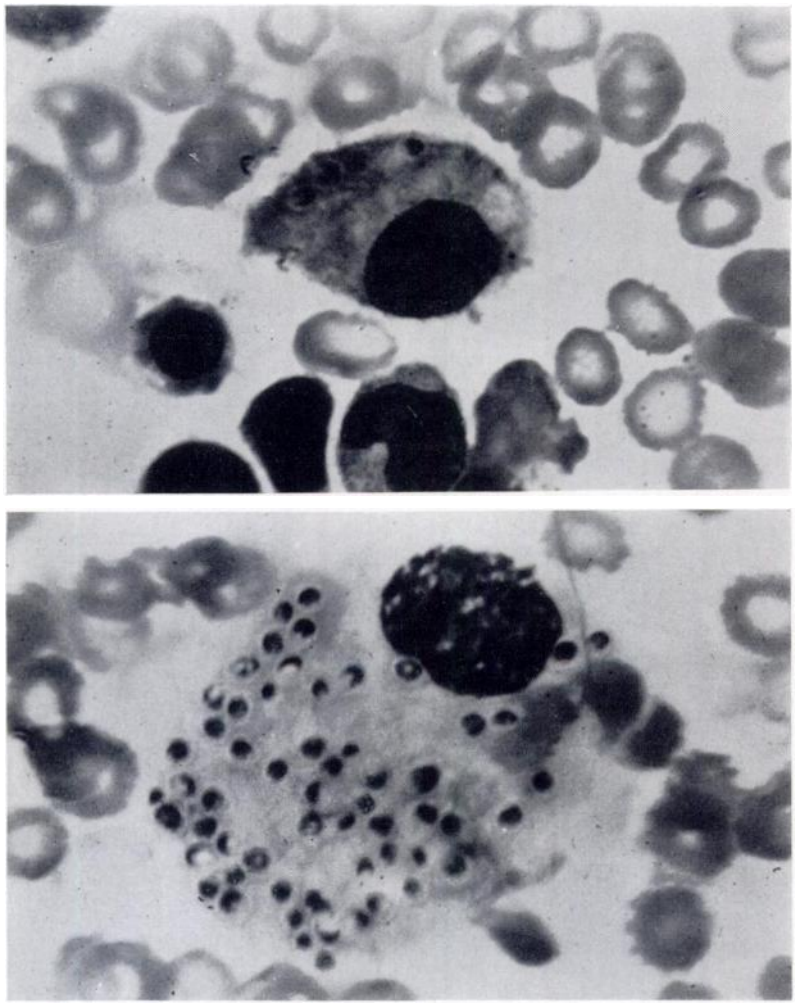

FIG. 1 *Top:* Plasma cell containing four *Histoplasma capsulatum* organisms in its cytoplasm. *Bottom:* Phagocytic clasmatocyte (histiocyte) which has engulfed large numbers of *H. capsulatum*. (From bone marrow of Case 1.)

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2 per cent stimulated monocytes. Total platelet count was 236,460. Bone marrow aspirate revealed a diminution in lymphocytes, increased numbers of young stimulated monocytes, and increased numbers of actively phagocytic clasmatocytes. Bone marrow culture yielded a growth of *H. capsulatum*. Complement fixation test for *H. capsulatum* was 3 plus in a 1:8 dilution. Histoplasmin skin test was positive in 1:100 strength. Chest x-ray showed a density extending from the hilum into the right lung field. After positive culture of marrow was obtained, patient was placed on ethyl vanillate therapy for forty-two days. After

this period of therapy patient was afebrile, anemia had disappeared and pulmonary infiltrations had completely cleared. Splenomegaly gradually disappeared over a longer period of time. When last seen in clinic on August 15, 1952 the patient was asymptomatic.

Case 3 (L. S.)

This 4½ month old white male infant was admitted to the James Whitcomb Riley Hospital on April 17, 1952 because of hepatomegaly and splenomegaly discovered by the family physician. There was a history of repeated upper respiratory infections for the past two months. Physical examination revealed temperature of 101.5 F., spleen palpable 4 cm. below the left costal margin and liver palpable 2½ cm. below the right costal margin. Hemogram was as follows: hemoglobin, 13.1 Gm.; total erythrocyte count, 6.66 million. Total leukocyte count was 3900 with a differential of 60 per cent polymorphonuclear neutrophils, 2 per cent neutrophilic "C" myelocytes, 30 per cent small lymphocytes and 8 per cent monocytes. Total platelet count was 466,200. Bone marrow examination revealed only pan marrow hyperplasia. Both the histoplasmin skin test and the complement fixation test for *H. capsulatum* were negative. X-ray of chest revealed an increased density in the right base. Patient was discharged without a definitive diagnosis on April 24, 1952. Because *H. capsulatum* grew in culture of bone marrow, he was re-admitted to the hospital on May 15, 1952 and given a course of ethyl vanillate therapy extending over thirty-three days. Bone marrow culture taken after twenty-two days of therapy was negative. While on therapy infant developed acidosis, anorexia and diarrhea. He expired on June 26, 1952. Permission for an autopsy was refused.

Case 4 (B. T.)

This patient was a 15 month old white girl who was admitted to the James Whitcomb Riley Hospital on June 9, 1952 because of fever and a pulmonary infection which had not responded to antibiotic therapy. Positive physical findings consisted of temperature of 104 F., slight hepatomegaly and splenomegaly. Hemogram revealed the following: hemoglobin, 7.8 Gm.; total erythrocyte count, 3.80 million. Total leukocyte count was 4550 with a differential of 83 per cent polymorphonuclear neutrophils, 1 per cent metamyelocytes, 11 per cent small lymphocytes, 4 per cent active monocytes and 1 per cent young monocytes. Total platelet count was 220,400. Examination of bone marrow aspirate revealed numerous *H. capsulatum* organisms free in the marrow and engulfed in phagocytic clasmatocytes, monocytes and polymorphonuclear neutrophils. Bone marrow culture also yielded *H. capsulatum*. Histoplasmin skin test was negative. Chest x-ray revealed multiple small densities scattered throughout the lung fields. Patient did not respond to treatment with ethyl vanillate and expired on the fourth day of therapy. Terminally, large numbers of *H. capsulatum* organisms could be seen in smears of peripheral blood. Autopsy confirmed the diagnosis of generalized histoplasmosis.

Case 5 (G. C.)

This 5½ year old white boy was admitted to the James Whitcomb Riley Hospital on May 11, 1952 because of intermittent low-grade fever, anemia, nausea and vomiting, and occasional frontal headaches; all symptoms were of three months' duration. Physical examination revealed temperature of 100 F., and soft systolic murmur along left sternal border. Chest was clear to percussion and auscultation. Liver and spleen were not palpable. Hemogram was as follows: hemoglobin, 11.6 Gm.; total erythrocyte count, 3.77 million. Total leukocyte count was 2800, with a differential of 64 per cent polymorphonuclear neutrophils, 2 per cent metamyelocytes, 18 per cent lymphocytes, 4 per cent intermediate lymphocytes, 2 per cent small Rieder cells, 8 per cent active monocytes and 2 per cent young monocytes. Total platelet count was 731,380. Bone marrow examination was not diagnostic, showing only pan marrow hyperplasia. Chest x-ray revealed enlarged left hilar lymph nodes. Tuberculin test was negative. Definitive diagnosis could not be made and patient was discharged on May 23, 1952. Several weeks following discharge bone marrow culture was found to be positive for *H. capsulatum*. Patient had moved to another

state and could not be readmitted to this hospital. Our last report on this patient was obtained on July 19, 1952 at which time he was said to still have intermittent low-grade fever and anemia.

Comment

Disseminated histoplasmosis is a disease of protean manifestations. It has been reported as occurring in association with various lymphomas.[8, 9] It may also mimic a wide variety of other hematologic entities.[10] Thus, in any infant or child who has any of the following findings: unexplained fever, lymphadenopathy, splenomegaly, hepatomegaly, and unexplained pulmonary findings with or without an abnormal peripheral blood picture, we have found it expedient to routinely culture the bone marrow for *H. capsulatum*.

In so doing we have obtained 5 positive cultures in 76 pediatric bone marrow cultures made during the two year period that we have provided hematology consultation service for the Pediatrics Department at the Indiana University Medical Center. This constitutes a positive yield of 6.5 per cent.

The culture media employed is universally obtainable and no special handling of the cultures is required. Care should be taken that cultures are not too quickly discarded. We have found that even in patients with a heavy infestation at least five days of growth are required. The average culture required ten to fourteen days for growth and occasional cultures did not become positive for four weeks.

It is our impression that there is some direct correlation between the severity of the disease, the time required for the organisms to grow out on culture and the prognostic outlook for the patient. This relationship was suggested by Klingberg.[3]

Of the 5 patients in whom positive cultures were obtained, 2 demonstrated *H. capsulatum* organisms on microscopic examination of the bone marrow specimen. These 2 patients had a rapid downhill course and expired in spite of all therapeutic efforts. In the remaining 3 patients, though the clinical suspicion of histoplasmosis was entertained, careful search of marrow specimens failed to yield any diagnostic features which set these apart from the majority of the 71 negative cases. In these 3, bone marrow culture was the single test which established the diagnosis.

While it is not within the province of this paper to discuss the value of any therapeutic agent, if the observations of Christie et al.[11] on the effectiveness of ethyl vanillate prove correct, it is in those patients who have minimal evidence of *H. capsulatum* that the most benefit can be expected.

Summary

1. Of 76 bone marrow cultures in pediatric patients with a variety of hematologic abnormalities, 5 (6.5 per cent) yielded *H. capsulatum*.

2. Methods used and brief case reports were described.

3. In 2 of the 5 cases with histoplasmosis, organisms were observed on direct examination of the marrow and cultures were only of confirmatory value.

4. In the remaining 3 patients with histoplasmosis, direct examination of marrow showed no features which distinguished them from the 71 negatives.

In these 3 bone marrow culture was the only means by which diagnosis could be established.

5. In no instance in which a negative bone marrow culture for *H. capsulatum* was obtained, was histoplasmosis diagnosed by other methods.

6. We are of the opinion that while routine marrow culture for *H. capsulatum* is of little value in adults, it is of considerable value for case finding in infants and children.

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