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Basic and Acidic Isoferritins in the Serum of Patients with Hodgkin's Disease

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Abstract—*Ferritin concentration has been measured in the serum of patients with Hodgkin's disease (HD) by radioimmunoassays with monospecific antibodies to liver (basic) and HeLa (acidic) ferritin. Elevated levels of serum ferritin with the liver ferritin assay were found only in patients with systemic disease, and were associated with low serum iron. Basic ferritin levels returned promptly to normal when complete remission was achieved. High levels of serum ferritin with the HeLa ferritin assay were found in 94% of all untreated patients. Acidic ferritin concentration was not related to systemic symptoms or alterations of iron metabolism, and returned to within the normal range only 1–2 yr after complete remission. These findings suggest that basic and acidic isoferritins can be distinguished in terms of biological and clinical significance. Basic ferritin is synthesized by the reticuloendothelial cells and the high values found in patients with systemic symptoms are compatible with the non-specific changes known to occur in the reticuloendothelial system during inflammation. In patients with untreated HD an elevated serum concentration of basic ferritin can be considered a marker of systemic symptoms and, therefore, an unfavourable prognostic factor. Acidic ferritin may be derived from abnormal lymphocytes and/or monocytes, including malignant cells, and its serum concentration may be of value in following the course of remission.*

INTRODUCTION

SEVERAL studies have shown the presence of abnormally high concentrations of serum ferritin in patients with Hodgkin's disease (HD), not related to the amount of body storage iron [1–10]. Relationships have been found between the serum ferritin concentration and both the clinical stage and prognosis, but such findings have not yet found clinical application [11].

In all the above studies serum ferritin concentration was measured using assays based on ferritin extracted from liver or spleen tissues. It has been shown that ferritin is present in tissues in multiple forms which differ structurally and immunologically [12–15]. These isoferritins are characterized by different pIs; the more basic ferritins, rich in L subunits, are predominant in spleen and liver, while the more acidic ones, rich in H subunits, are predominant in heart, erythrocytes, lymphocytes, monocytes and some tumour tissues, such as HeLa cells [16].

It has been found that HD-involved spleens contain more acidic isoferritins than normal spleens [17]. More recently, Dörner *et al.* [18] have shown that T lymphocytes from the spleen of HD patients synthesize and secrete a very acidic ferritin molecule. Therefore such acidic isoferritins might also be present in the serum of patients with HD. However, the commonly available immunoassays for serum ferritin are based on liver or spleen ferritin and greatly underestimate acidic ferritin types [13, 15].

In the present work we have measured ferritin concentration in the serum of patients with HD by means of radioimmunoassays with monospecific antibodies to liver and HeLa ferritin. For

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simplicity of writing, we shall use the terms basic and acidic ferritin for defining the ferritin types measured by the liver and HeLa ferritin assay respectively. The results obtained show that high serum concentrations of both basic and acidic isoferritins can be detected in HD and suggest that such isoferritins may be distinguished in terms of biological and clinical significance.

MATERIALS AND METHODS

Patients

Eighty-seven consecutive patients with HD seen from July 1976 to June 1981 were evaluated. The patients included 50 males and 37 females and were 13–66 yr old. Fifty-one subjects had untreated disease at the time of our observation, and 8 of them could also be evaluated in complete remission (CR) after the halt of any therapy (one subject was evaluated two times in CR); 1 patient was re-evaluated in partial remission (PR). Twelve patients were in CR, 4 in PR and 20 in relapse at the time of our observation; 2 of the patients in CR were re-evaluated later in CR, while 5 of them were studied also in relapse. A total of 104 serum samples were therefore analysed.

Histological type and clinical stage of HD were evaluated according to Ann Arbor criteria [19–21]; 36/87 patients underwent pathological staging. Complete remissions were judged by Carbone and Spurr's categories [22] and were guaranteed by a disease-free period of at least 6 months.

Fifty-four healthy subjects were examined at the time that routine blood samples were being taken for screening purposes. They were well matched with respect to age and sex.

Laboratory methods

Haematological data were obtained by standard techniques [23]. Serum iron, TIBC and copper levels were determined by atomic absorption spectrophotometry [24]. Radioimmunoassays for liver and HeLa ferritins were developed as previously described in detail by Arosio *et al.* [25]. Serum samples were assayed in duplicate, while standards were assayed in triplicate. Sera were stored at -20°C until used. Immunoreactivity with both liver and HeLa antibodies did not change with time in storage, but it was of critical importance that the serum had been depleted of complement. In fact, complement appeared to influence the immunoassay with HeLa antibodies, producing falsely high values for serum ferritin concentration in some cases. Addition of EDTA to the test tube removed this interference.

RESULTS

Table 1 summarizes the values obtained with the 2 ferritin assays. There was no correlation between the paired values for basic and acidic ferritin concentration. Basic ferritin levels were higher in males than in females, and in subjects over the age of 40 yr than in younger ones. On the contrary, acidic ferritin levels were not related either to sex or age.

Basic ferritin concentration was significantly increased in HD patients at presentation and in relapse with respect to normal controls ($P<0.001$). Acidic ferritin concentration was increased in most of the HD patients and no major differences between the different phases of the disease could be found by considering the mean values (Table 1).

Table 1. Serum ferritin concentrations in normal subjects and patients with Hodgkin's disease

Subjects (No.)	Basic ferritin concentration (liver ferritin assay) Geometric mean (range) (μg/l)	Basic ferritin concentration (liver ferritin assay) % of patients with ferritin > the upper normal limit	Acidic ferritin concentration (HeLa ferritin assay) Geometric mean (range) (μg/l)	Acidic ferritin concentration (HeLa ferritin assay) % of patients with ferritin > the upper normal limit
Normal subjects (54)	76 (18–251)		16 (5–41)	
Patients with HD:				
at presentation (51)	175 (12–2100)	37	103 (30–1000)	94
during CR (23)	67 (14–230)	0	72 (21–570)	74
during PR (5)	77 (27–165)	0	188 (145–220)	100
in relapse (25)	143 (31–1268)	36	131 (25–750)	88

Figure 1 shows the ferritin measurements in the 2 subgroups of patients at presentation, without (A) and with systemic symptoms (B). Basic ferritin concentration was normal in almost all the patients without systemic symptoms and was abnormally elevated in most of the patients with systemic symptoms. On the other hand, acidic ferritin concentration was abnormally elevated in nearly all patients and did not appear to be influenced by the presence or absence of systemic symptoms.

Basic ferritin concentration was higher in stages III and IV than in stages I and II, and in the more unfavourable histological types (mixed cellularity and lymphocyte depletion) than in the more favourable ones (lymphocyte predominance and nodular sclerosis). However, these differences were due to unequal distribution of patients with systemic symptoms between stages and histological types. Basic ferritin concentration was unrelated to ESR, Hb level, alpha-2-globulin level, serum copper concentration, fibrinogen concentration and lymphocyte and monocyte counts. It was inversely related to serum iron concentration in patients with systemic symptoms at presentation (Fig. 2).

Acidic ferritin concentration was unrelated to either clinical stage or histological type, and also to any of the above-mentioned parameters, including serum iron. However, in patients in CR the acidic ferritin concentration appeared to be related to the remission duration (Fig. 3). It was

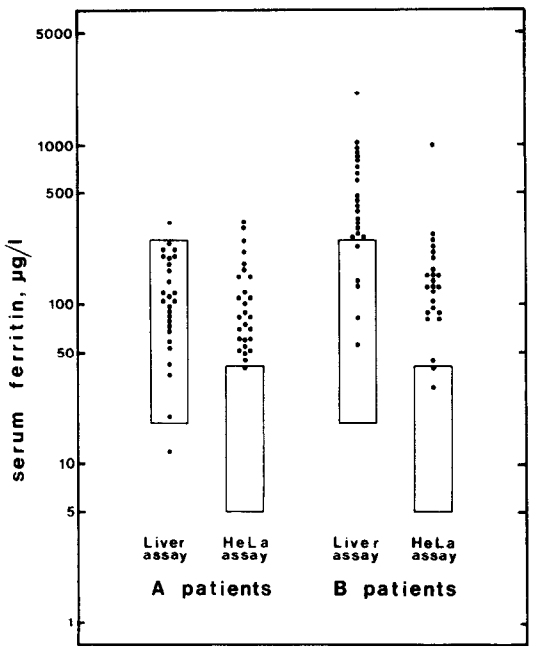

Fig. 1. Basic (liver assay) and acidic (HeLa assay) ferritin concentrations in the serum of patients with untreated HD. A refers to patients without, B with systemic symptoms. Rectangles represent the normal range.

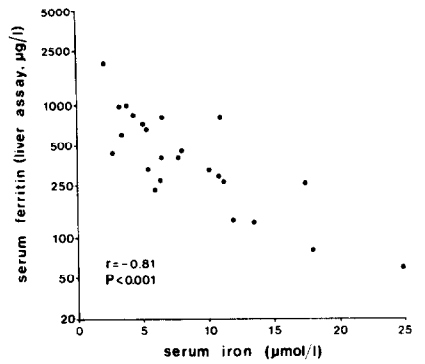

Fig. 2. Relationship between serum iron and basic (liver assay) ferritin concentration in patients with untreated HD and systemic symptoms (B patients).

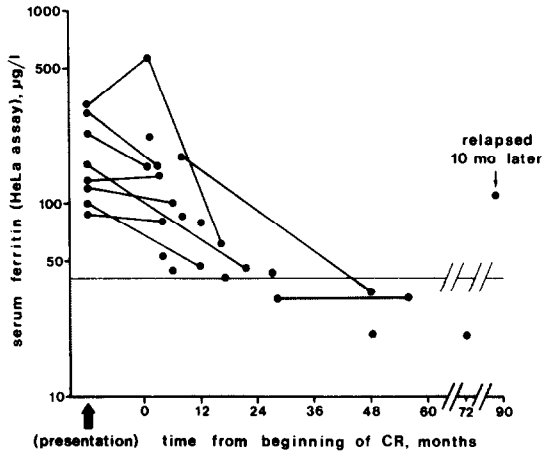

Fig. 3. Relationship between duration of complete remission and serum concentration of acidic ferritin (HeLa assay) in patients with HD. Lines join sequential determinations in a single patient. The shaded area represents the normal range for acidic ferritin concentration.

still high at the beginning of CR (while the basic ferritin concentration was normal at this time) and returned to within the normal range in about 1–2 yr. All the patients tested after 30 months of CR had a normal acidic ferritin concentration, with one exception: a patient who relapsed 10 months later. This woman had mixed cellularity HD, clinical stage III_S B. At the time of our observation during CR, the basic ferritin concentration was normal (50 µg/l) while the acidic ferritin concentration was high (110 µg/l).

DISCUSSION

It is now well established that serum ferritin concentration, as measured by a liver or spleen ferritin assay, is tightly related to body iron stores in adult healthy subjects and in patients with various disorders of iron metabolism [11]. However, conditions such as liver disease, infection, inflammation and malignancy may elevate serum ferritin to a degree disproportionate to that of storage iron [11, 26].

Several factors may be responsible for ferritinemia in malignancy [11]: (a) malignant cells may produce large amounts of ferritin and secrete it into the plasma; (b) reticuloendothelial cells may increase ferritin synthesis [27]; (c) there may be leakage of ferritin from damaged cells (tissue necrosis); (d) metastatic liver involvement may produce a reduction in the rate of clearance and an increase in serum ferritin levels. Some evidence indicates that at least some of these factors actually play a role in HD.

Evidence has been obtained in previous studies [6, 7, 28, 29] that in patients with HD, high serum concentrations of basic ferritin are associated with low serum iron, reduced transferrin saturation and depression of marrow erythroid activity. These findings are compatible with the non-specific changes known to occur in the reticuloendothelial cells of all patients with chronic disease [27]. The present study confirms previous reports. The inverse relationship between serum iron and basic ferritin found by us (Fig. 3) is in agreement with the hypothesis of the iron reticuloendothelial block [27]. Considering that none of the untreated patients had liver abnormalities, it may be concluded that the elevated levels of basic ferritin were essentially due to an augmented synthesis by the reticuloendothelial cells. From a clinical standpoint, an elevated level of basic ferritin associated with a low serum iron concentration can be considered a sign of systemic symptoms and, therefore, an unfavourable prognostic factor.

A number of experimental studies suggest that other sources of serum ferritin are active in patients with HD. Sarcione *et al.* [30, 31] found increased ferritin synthesis and release by HD splenic tumor tissue and peripheral blood lymphocytes. The presence of intracellular ferritin deposits in HD peripheral blood lymphocytes has been confirmed by ultrastructural studies [32]. Functional T-lymphocyte defects have been shown in HD [33, 34], and it has been found that a subpopulation of peripheral blood T lymphocytes do not form E rosettes: this subpopulation can be unblocked by levamisole and this causes ferritin to be shed from the surface of the so-treated cells [35, 36]. A subpopulation of T lymphocytes (13–25%) bearing surface ferritin has been demonstrated in patients with HD [37].

All the above observations indicate that HD T lymphocytes synthesize and probably secrete more ferritin than do normal lymphocytes. According to the findings of Dörner *et al.* [18], this ferritin should be predominantly acidic, i.e. very similar to HeLa ferritin and different from the basic ferritin synthesized by the reticuloendothelial cells.

The results obtained in the present study by testing sera from normal subjects and patients with Hodgkin's disease with the HeLa ferritin assay indicate that: (a) acidic isoferritins are present in normal sera; (b) acidic and basic isoferritins may have different biological functions and clinical significance; (c) elevated levels of acidic ferritin are present in the serum of patients with HD.

In normal subjects the mean value for acidic ferritin concentration was about one-fifth that for basic ferritin concentration (Table 1). However, our values are relatively high when compared to those of Jones *et al.* [38]: in their study, HeLa-type ferritin was not detected (<2 $\mu g/l$) in most normal sera. On the other hand, Niitsu *et al.* [39] studied a small group of normal subjects using a heart ferritin assay and found values which are higher than ours. Most of these discrepancies are probably due to methodological factors since there are many difficulties in producing antibodies to acidic ferritins, and different antibodies may provide consistently different results [40]. Theoretically, since acidic ferritins are predominant in some tissues (heart) and some peripheral blood cells, such as erythrocytes, lymphocytes and monocytes [16], it is possible that they are released into the circulation and that low levels can be detected in the serum. Actually, Halliday *et al.* [41], using sensitive electrofocusing techniques, showed the presence of acidic isoferritins in normal serum. Worwood and co-workers (see review by Worwood [16]) also found these acidic ferritins, but they were unable to detect them with an immunoradiometric assay for human heart ferritin. They concluded that much of the heterogeneity of serum ferritin, demonstrable by isoelectric focusing, is due to the presence of sialic acid residues and not to variation in subunit composition. This controversy on the presence of acidic isoferritins (rich in H subunits) in normal serum will be resolved only by the development of monoclonal antibodies specific for H subunits.

The biological significance of both basic and acidic isoferritins in the serum is far from clear. Nevertheless, basic ferritin appears to be related to iron metabolism [11]. On the contrary, the few available data on acidic isoferritins in serum, including those of the present report, do not show any correlation with iron metabolism. Interestingly, Broxmeyer and co-workers [42] have recently demonstrated that acidic isoferritins play a role in the regulation of normal myelopoiesis, showing an inhibitory activity.

Acidic ferritin concentration in serum was high in most of the patients with HD, and appeared to return to within the normal range slowly (Fig. 3).

Obviously, no firm conclusion can be drawn from these data since only small numbers of patients in remission were studied and few sequential studies in single patients were carried out. Nevertheless, the slow normalization time of acidic ferritin concentration is in agreement with the time course of T-lymphocyte deficiencies during remission of HD [43, 44]: usually, some years elapse before lymphocyte function becomes normal. This finding would implicate a connection between acidic ferritin and T lymphocytes, thus supporting the results obtained by Dörner *et al.* [18]. This does not exclude other sources of acidic ferritin in HD, which may be represented by monocytes and cells of the malignant tissue [30]. If the data reported in Fig. 3 is confirmed by sequential studies which are presently undertaken in our departments, the HeLa ferritin assay might become a useful tool for monitoring complete remission of HD.

Recent studies have indicated that the serum ferritin assay with antibodies to human liver or spleen ferritin may provide a tool of potential diagnostic and prognostic importance in the management of some malignancies [8, 45]. Interesting results have been obtained using an assay with antibodies to human placental ferritin, which have a broad specificity against most human isoferritins [46, 47]. The use of assays specific for basic and acidic isoferritins might allow even better results to be obtained.

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