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LETTER TO EDITOR

IFN-g:IL-10 Ratio: a Putative Predictive Biomarker to Discriminate HLH From Severe Viral Infections

Snehal Shabrish[1] · Mukesh Desai[2] · Vinay Saxena[3] · Madhura Kelkar[1] · Manisha Madkaikar[1]

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To the Editor,

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening, hyperinflammatory syndrome, caused due to immune dysregulation. HLH may occur as a primary (genetic) condition due to mutations in genes important in the pathway of granule-mediated cytotoxicity or as a secondary condition where identical clinical findings may arise secondary to infectious, rheumatologic, malignant, or metabolic conditions. Preliminary diagnosis of HLH is based on guidelines of the Histiocyte Society [1] which includes clinical manifestations and laboratory investigations. These criteria are useful for HLH diagnosis especially when the definitive genetic diagnosis is absent. However, several diagnostic challenges still prevail; one of which is differentiating severe infection from HLH precipitated by infection. Viral infections are frequently implicated at the onset of active HLH episodes. Viral infections are also known to have an impact on NK cell and cytotoxic T cell (CTL) function and induce cytokine release leading to inflammatory conditions; however, not all viral-infected patients develop HLH.

Hypercytokinemia is a hallmark of HLH and is responsible for most clinical and laboratory findings. Studies have shown that Th1 and Th2 cytokines, regulating immune responses, increase significantly and play a critical role in the pathogenesis of HLH [2]. Measurement of cytokine levels is currently not routinely performed in clinical settings; however, few studies suggest that incorporation of rapid cytokine profiling may be extremely helpful in facilitating diagnosis of HLH. Also, though few studies report difference in cytokine pattern between HLH patients and non-HLH with infections, to the best of our knowledge, none of these reports have compared the cytokine profile in patients with viral infection and viral triggered secondary HLH (2° v-HLH). The advent of bead-based flow cytometry assay for cytokine level determination will help in prompt identification of patients susceptible to develop HLH. Thus, in this study, we compared cytokine and chemokine pattern of patients with secondary HLH induced by viral infection (2° v-HLH) ($n = 7$), non-HLH viral-infected (v-non-HLH) patients ($n = 10$), and familial HLH (FHL) ($n = 6$) (2 FHL2, 3 FHL3, and 1 FHL4) to determine the difference in cytokine pattern leading to development of HLH.

HLH patients were diagnosed based on HLH criteria of the Histiocyte Society [1] and patients with identified mutations were considered FHL while the remaining patients were 2°-HLH. Patients presenting with fever but not fitting into HLH criteria were considered as non-HLH. 2° v-HLH and non-HLH patients were screened for viral infections using FTD Neuro9 kit (Fast Track Diagnostics Luxembourg) as per manufacturer's instructions, and those patients with viral infection were included in this study as 2° v-HLH and v-non-HLH respectively. Plasma samples from all these patients were collected during acute phase of the disease and all these patients were treatment naïve at the time of sample collection.

Cytokines essential for inflammatory response (IFN-γ, TNF-α, IL-12p40), initiating humoral response (IL-4, IL-6, IL-10), cellular growth (GM-CSF, IL-2, IL-7, IL-15), and chemokines (IP-10, MIP-1α, MIP-1β, hMCP-1) were evaluated using AIMPLEX kit (as per manufacturer instructions). Median and range for all the parameters was calculated. Kruskal-Wallis test and Mann-Whitney *U* test were used to compare data between the groups. Spearman correlation was performed to analyze the correlation between variables. Analyses were performed with GraphPad Prism (GraphPad

✉ Manisha Madkaikar
madkaikarmanisha@gmail.com

[1] Department of Pediatric Immunology and Leukocyte Biology, National Institute of Immunohaematology (ICMR), 13th floor, Multistoreyed Building, KEM Hospital Campus, Parel, Mumbai 400012, India

[2] Division of Immunology, Bai Jerbai Wadia Hospital for Children, Parel, Mumbai 400012, India

[3] National Institute of Virology, Mumbai Unit, Haffkine Institute Compound, Acharya Donde Marg, Parel, Mumbai 400012, India

Software, Inc. Version 5.0). Differences were considered statistically significant if the 2-tailed p value was ≤ 0.05.

Consistent with previous studies, the current study also demonstrated a significant increase of serum levels of cytokines and chemokines in HLH patients compared with healthy controls ($p < 0.05$). HLH patients present a specific cytokine profile of highly increased levels of IFN-γ and IL-10, and a moderately increased level of IL-6 [2], which was also seen in our patients. In our study, FHL patients (179.95 pg/ml; 4.91–923.32 pg/ml) had significantly elevated IFN-γ levels ($p < 0.05$) when compared to 2° v-HLH patients (15.45 pg/ml; 3.3–111.94 pg/ml), whereas the other cytokines were comparable. In the report by Chen et al. (2016), IL-4 level in primary HLH was significantly lower than that in secondary HLH ($p = 0.025$), while IFN-γ level in primary HLH was statistically lower than that in secondary HLH ($p = 0.051$) [2]. However, they had included only 4 primary HLH patients (1 FHL2 and 3 XLP-1) and some of the secondary HLH patients had single heterozygous mutations in HLH-related genes. Thus, the variation in our findings could be due to a difference in study population.

Cytokines not only promote proliferation but also inhibit the differentiation and function of each other to maintain immune homeostasis. Compared to healthy controls, cytokine profile in HLH patients as well as v-non-HLH patients showed significant increase in IFN-γ and IL-10 and significantly reduced IFN-γ:IL-10 ratio ($p < 0.05$). Interestingly, though 2° v-HLH and v-non-HLH patients had similar cytokine pattern, the former had significantly elevated IFN-γ:IL-10 ratio (0.73; 0.19–2.3) compared to the latter (0.09; 0.03–0.3) ($p = 0.003$). No significant difference between FHL and 2° v-HLH in terms of IFN-g/IL-10 ratio was observed. IFN-γ is a Th1 cytokine that is produced in response to inflammatory stimuli to regulate the human immune response. IL-10, on the other hand, has strong immunosuppressive properties, and acts as a potent inhibitor of Th1 effector mechanisms. IL-10 acts as an inhibitory cytokine blunting NK cell activation, which restrains IFN-γ secretion and has a significant effect on NK cell cytotoxicity [3]. The level of these cytokines determines immune regulation and their overproduction plays an important role in the pathophysiology of FHL [2]. Recently, An et al. (2017) reported association of polymorphism in IFN-γ and IL-10 genes with HLH susceptibility [4], thus highlighting the role played by these cytokines in HLH pathogenesis. So, though post-infection IFN-γ as well as IL-10 levels increases, rather than alone, IFN-γ/IL-10 ratio is more informative in HLH diagnosis (Fig. 1a–c).

ROC curve was generated based on the IFN-g/IL-10 ratio for HLH and v-non-HLH patients. Area under the curve for IFN-g/IL-10 ratio was 0.868 (95% CI, 0.7342 to 1.020)

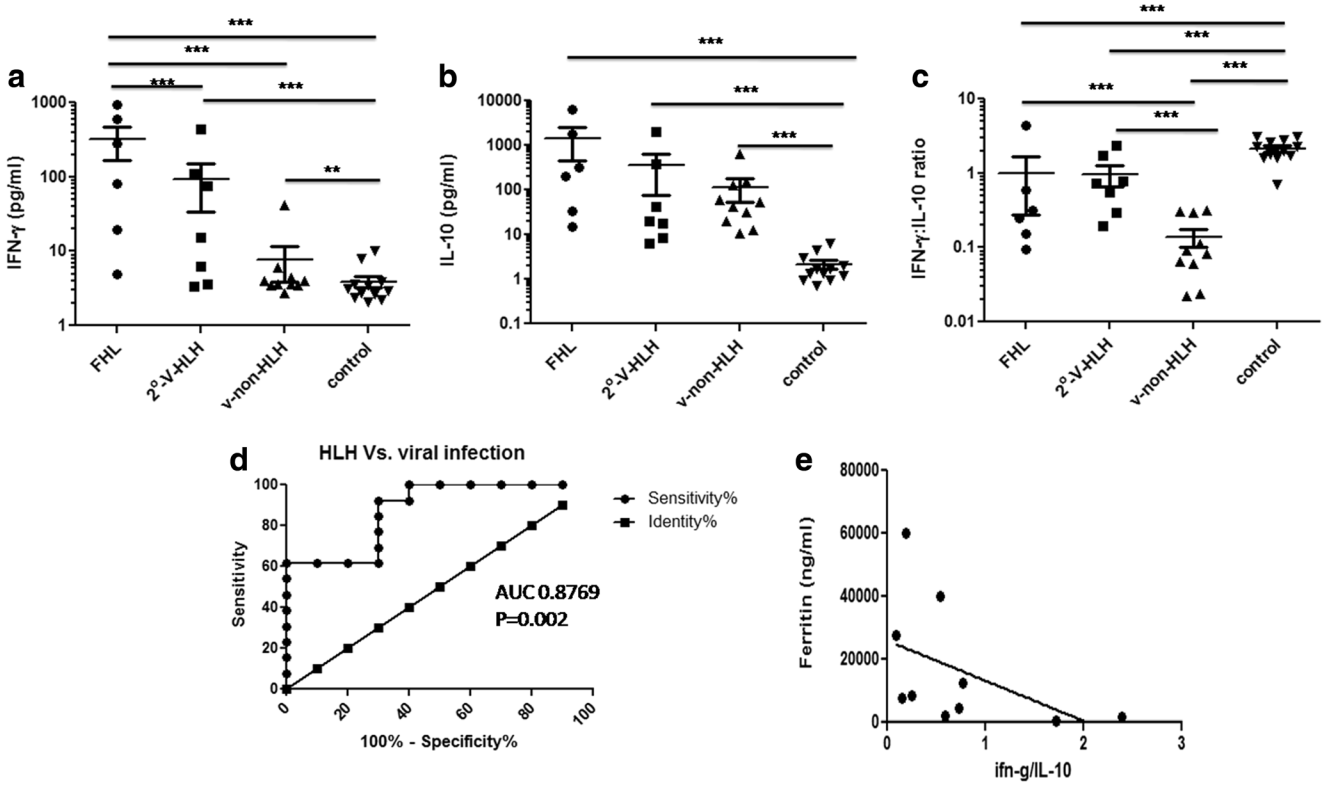

Fig. 1 Comparison of serum cytokine level scatter plot graph and the line at the middle is the mean. ** $p < 0.05$, *** $p < 0.01$. **a** IFN-γ levels. **b** IL-10 levels. **c** IFN-γ:IL-10 level ratio. **d** ROC curve showing abilities of IFN-γ:IL-10 level ratio in predicting HLH from severe viral infections. **e** Correlation of IFN-γ:IL-10 level ratio with ferritin levels

indicating that IFN-g/IL-10 ratio was significant discriminator for patients with HLH ($p = 0.0023$). IFN-γ/IL-10 ratio cutoff of 0.133 (with the optimal Youden index) showed a sensitivity of 92.31%, specificity of 70%, and a total accuracy of 82.61% for diagnosis of HLH (Fig. 1d).

Hyperferritinemia is an important diagnostic as well as prognostic biomarker of HLH and has high sensitivity and specificity. It also correlates with disease activity. In our study, IFN-γ:IL-10 ratio in HLH patients negatively correlated with ferritin levels ($r = -0.4824$, $p = 0.158$) (Fig. 1e). However, correlation was not significant which could be attributed to small sample size and needs to be evaluated in a larger cohort. We believe this ratio may serve as an additional predictive biomarker for differentiating HLH from severe infections.

Generally, IFN-γ contributes to inflammatory response while IL-10 is claimed to be protective and immune modulator. In HLH, all reports address the roles of cytokines individually [2]. However, in the present study, we inferred that to determine the pathogenesis of HLH, IFN-γ /IL-10 ratio is more informative than interpreting either cytokine alone. These conclusions, however, are drawn from a relatively small number of cases. Thus, we suggest that validating these results in a larger cohort might help in verifying if this pattern could possibly predict the susceptibility to develop HLH in viral-infected patients.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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