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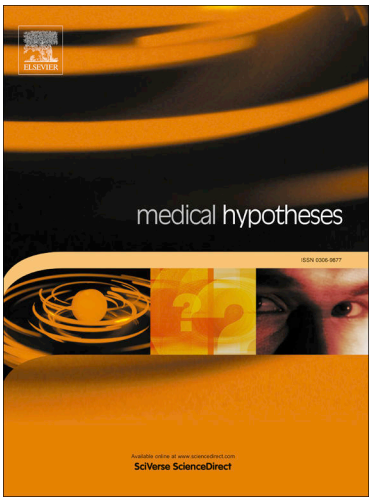

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Etoposid-based therapy for severe forms of COVID-19

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Abstract

The new coronavirus infection COVID-19 has quickly become a global health emergency. Mortality is principally due to severe Acute Respiratory Distress Syndrome (ARDS) which relays only on supportive treatment. Numerous pathological, clinical and laboratory findings rise the similarity between moderate to severe COVID-19 and haemophagocytic lymphohistiocytosis (HLH). Etoposide-based protocol including dexametasone is the standard of care for secondary HLH. The protocol has been successfully used in HLHs that are secondary to EBV and H1N1 infections by inducing complete response and pronged survival. These observations prompt to consider this cytotoxic therapy in HLH associated to moderately severe to severe forms of COVID-19.

Key words: COVID-19; Haemophagocytic lymphohistiocytosis; HLH; Etoposide

Contributors

K H and S A considered HLH treatment. G B supported this idea and developed it further. G B wrote the manuscript and the final version was approved by all the authors.

Conflict of interest statement

We declare that we have no conflict of interest.

Introduction

The COVID-19 was declared first by the World Health Organization (WHO) as a public health emergency of international concern then it was characterized as a pandemic (1). The health crisis has led to another deep and global socio-economic crisis. This rises the need to urgently find a treatment in order to reduce the length of stay in hospitals and intensive care units and the number of deaths. Since vaccines are still at least 12 to 18 months away, the focus is on drug development by exploring the available therapeutic possibilities.

Severe cases are the most challenging as they may be complicated by a severe Acute Respiratory Distress Syndrome (ARDS) (2). The current management of ARDS is only supportive (3). But there are many ongoing clinical trials testing potential antiviral drugs that have been used against Betacoronaviruses associated with previous epidemics of SARS-CoV and MERS-Cov (HIV drugs or Ebola frizzled drug) as well as promising malaria drugs chloroquine and hydroxychloroquine.

COVID-19 and haemophagocytic lymphohistiocytosis (HLH)

The development of ARDS in COVID-19 is associated to the upregulation of many pro-inflammatory cytokines and chemokines: interleukin (IL)-2, IL-7, granulocyte colony stimulating factor, interferon-γ (IFN-γ), inducible protein 10, monocyte chemoattractant protein 1, macrophage inflammatory protein 1-α, and tumour necrosis factor-α (TNF-α) (4). This up-regulation is the main characteristic of Cytokines Storm Syndromes (CSS). The CSSs are associated with hemophagocytic lymphohistiocytosis (HLH) which can be primary or familial (pHLH) or secondary, acquired or reactive (sHLH) (5). The sHLH, occurring frequently after an infection, are life-threatening syndromes of extreme immune activation leading to a multiorgan failure and a severe hypercytokinaemia (6). The revised criteria for HLH diagnosis include fever, splenomegaly, bicytopenia, hypertriglyceridaemia or hypofibrinogenaemia (or both), haemophagocytosis, ferritin ≥500 µg/L, low NK cell activity, and soluble IL-2 receptor ≥2400 U/mL). Five of the eight criteria in total are needed to make a diagnosis of HLH **(Table 1)** (7). It is noteworthy that many of these criteria were described as predictors of COVID-19 mortality(7-9). Moreover, pathologic examinations of a COVID-19 patient's lung revealed patchy inflammatory cellular infiltration and multinucleated giant cells (10). The latter cells may be haemophagocytes.

Even though the pathophysiology of HLH secondary to an infection is still largely unclear, it is presumably similar to the one of pHLH (5). In immunocompetent individuals, intracellular pathogens trigger a T-helper cell 1 (Th1)-type immune response with a release of pro-inflammatory cytokines that activate histiocytes (macrophages and dendritic cells), NK cells and cytotoxic T-cells (CTLs). These cells continue to reciprocally stimulate each other through receptor interaction and by cytokines. In sHLH, there is a dysfunction of CTLs and/or NK cells leading to the persistence of the antigenic insult which maintains cytokines release (11). Interestingly, data from China suggests the viral load is higher in patients with more severe disease (12). This may be due the persistence of the viral insult caused by cytotoxic cells dysfunction.

Clinical and laboratory findings in HLH can be explained by the rise of pro-inflammatory cytokines, the organ infiltration by activated lymphocytes and histiocytes, and the hypofibrinogenemia resulting from increased plasminogen activator expressed by activated macrophages (7). We can notice that many of these findings have been described in patients with severe COVID-19 (2, 4). Besides, it was described that the median time from onset of COVID-19 symptoms to ARDS is 9·0 days (8·0–14·0) (4). This is consistent with observations made in the context of HLH development during H1N1 infection (13). Hence, there are accumulating elements suggesting that moderately severe to severe COVID-19 might be a form of sHLH.

Could sHLH treatment be applied on COVID-19?

Etoposide-based treatment is the standard of care for sHLH following the Consensus Statements by the HLH Steering Committee of the Histiocyte Society. The treatment aims at dampening the cytokine storm and is based on etoposide and dexamethasone with intrathecal methotrexate in case of central nervous system involvement (14, 15).

Interestingly, this treatment induced complete remissions in moderately severe to severe sHLH associated to EBV Virus especially when it was started less than 4 weeks from infection diagnosis (16-18). The combination has also been reported to be of value in severe influenza A/H1N1 and hepatitis-B-virus associated HLH (19, 20). It is possible that the limited use of this protocol in sHLH associated to other viral infections may be due to their rarity or to their under-diagnosis.

Etoposide is an ancient cytotoxic agent with predictable side effects that can be symptomatically treated. It may also induce secondary malignancies especially acute myeloid

leukaemia. This risk is estimated at 0·3–0·4% (21). However, we think that it can be balanced by the risk of mortality and of neurological sequelae in severe forms of COVID-19. It would be possible also to propose this treatment to COVID-19 patients who are already suffering from a cancer. This may at least reduce the mortality in this fragile sub-group. Additionally, it should be noticed that Etoposide is a widely used and a relatively cheap medication. Hence, we think that it should be tested in moderately severe to severe cases of COVID-19 especially since the pandemic is rapidly progressing with a non-neglectable mortality.

The expected therapeutic mechanism of Etoposid

Etoposide is a topoisomerase II inhibitor. It has been shown, in a murine model of HLH, that its therapeutic mechanism involved potent deletion of activated T cells and efficient suppression of inflammatory cytokine production. This was a remarkably selective effect; no direct anti-inflammatory effect on macrophages or dendritic cells was observed and no deletion of quiescent naive or memory T cells (22). This effect does not seem to hamper antiviral response in human since the combined therapy with dexamethasone increased the survival of patients with EBV associated HLH (16-18).

How could the treatment be tested?

The HLH Steering Committee of the Histiocyte Society recommends the HLH-94 protocol as standard of care for infection-Associated HLH(15). The suggested therapy for patients with viral infections and severe sHLH is the use of the combination of etoposide and dexamethasone for eight weeks. Age-adjusted doses of Etoposide are administered once a week with weekly decisions on whether to continue etoposide treatment or not, following the clinical and laboratory response of the patient. Dexamethasone is administered daily starting with 10mg/m² during the first week then the dose will half decrease every week. It is important to keep antivirals and to provide supportive care: broad-spectrum antibiotics, antimycotic and gastric protection (19).

The authors submitted the present protocol to the Ministry of Population Health and Hospital Reform in their region and are waiting for its eventual adoption. Alternatively, we think that WHO may consider this protocol in the ongoing clinical trials on COVID-19 treatment.

Table 1: Secondary HLH diagnosis criteria

1. Fever
2. Splenomegaly
3. Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood): Hemoglobin <90 g/L (in infants <4 weeks: hemoglobin <100 g/L) Platelets <100x10^9/L Neutrophils <1.0x10^9/L
4. Hypertriglyceridemia and/or hypofibrinogenemia: Fasting triglycerides ≥3.0 mmol/L (i.e., ≥265 mg/dl) Fibrinogen ≤1.5 g/L
5. Hemophagocytosis in bone marrow or spleen or lymph nodes. No evidence of malignancy
6. Low or absent NK-cell activity (according to local laboratory reference)
7 Ferritin ≥500 mg/L
8. Soluble CD25 (i.e., soluble IL-2 receptor) ≥2,400 U/ml

Secondary HLH diagnosis criteria adapted from Henter et al. 2007. Five out of the eight criteria should be fulfilled.

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