Review Articles

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Research Developments on Risk Prediction Models and Risk Factors for Early-Onset Hemophagocytic Syndrome Associated with Epstein-Barr Virus Infection in Children

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ABSTRACT

Children with hemophagocytic lymphohistiocytosis (HLH) triggered by Epstein-Barr virus (EBV) have a poor prognosis, and early detection is essential for improving outcomes. Genetic sequencing is constrained by limited turnaround time, and classical diagnostic criteria often apply only at advanced disease stages. Consequently, there is an urgent need to examine the trajectories of multidimensional clinical signs and biochemical and immunological risk factors during the very early phase of illness. Current research is progressively overcoming the limitations of single, static marker analysis by integrating multiple high-risk clinical features from early disease stages into machine learning algorithms. This paradigm shift toward high-precision, dynamic early-risk prediction models addresses the traditional challenges of differential diagnosis in emergency settings and lays the foundation for individualized, precision management of critically ill children at high risk. This article provides a comprehensive review of recent advances in risk factor identification and risk prediction model development for early-onset EBV-associated HLH in children.

1. Introduction

Epstein-Barr virus (EBV) is a double-stranded DNA virus belonging to the Gammaherpesvirinae subfamily of the Herpesviridae family. Over 90% of the global population is infected with EBV, which is primarily transmitted through saliva [1]. In children under six years of age, EBV infection frequently produces no symptoms or only mild upper respiratory manifestations. By contrast, approximately 50% of adolescents may develop self-limiting infectious mononucleosis (IM), reflecting differences in immunological status. In a small subset of children, however, EBV infection triggers a particularly severe multisystem complication: hemophagocytic lymphohistiocytosis (HLH).

HLH is a pathogenic immune activation syndrome characterized by immune dysregulation and systemic hyperinflammation. It is responsible for more than 70% of secondary HLH cases in children [2], underscoring the central role of EBV in pediatric HLH pathogenesis. The disease is marked by rapid progression, high mortality, and diagnostic difficulty. No specific early warning signs exist, and early identification of EBV-HLH relies heavily on clinician experience and application of the HLH-2004 diagnostic criteria [3]. Multicenter studies have demonstrated that although outcomes for pediatric EBV-associated HLH are more favorable than for adults, the case-fatality rate among children who do not receive chemotherapy is extremely high [4], whereas the 6-month cumulative survival rate approaches 90.2% with appropriate treatment [5].

It is therefore imperative to identify children at high risk for HLH among the large population of febrile pediatric patients with EBV infection, and to accurately estimate prognosis following diagnosis to guide risk-stratified therapy. This article comprehensively reviews research from the past five years on early-stage risk factors and the development of risk prediction models for this disease. It examines the principal influencing factors and key findings in children with early-stage EBV-associated HLH, with the aim of providing a reference for future development of high-precision early-warning models driven by multidimensional clinical data, optimization of early risk stratification, and construction of personalized precision intervention systems.

2. Overview of Risk Prediction Models

A risk prediction model is a mathematical tool that integrates multiple predictors and their corresponding weights to estimate the probability that an individual will experience a specific outcome, either currently or in the

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future [6]. Risk prediction models are widely applied in clinical research to assist physicians in disease identification and patient risk stratification, thereby facilitating the transition from empirical medicine to precision medicine.

Clinical prediction models can be broadly categorized into diagnostic models and prognostic models, depending on the specific prediction objective [7]. These models typically rely on defined predictive variables, including multidimensional data such as demographics, clinical symptoms, laboratory measurements, and imaging features. Commonly employed modeling techniques include logistic regression and Cox proportional hazards regression [8][9]. With the expansion of medical big data and advances in computational capacity, machine learning algorithms offer distinct advantages in handling high-dimensional data and capturing nonlinear relationships and complex interactions among variables [10]. In the context of predictive diagnosis and management of pediatric EBV-associated HLH, models are frequently constructed using methods such as LASSO regression, random forests, support vector machines, and XGBoost. Ensuring model stability and generalizability requires rigorous validation during model development [11]. Model performance is evaluated using the area under the receiver operating characteristic (ROC) curve (AUC) for discrimination, calibration curves for calibration, and clinical decision curves for clinical utility [12]. Some studies provide visual model interpretations through scoring tables, nomograms, or web-based risk calculators, whereas others rely on regression coefficients for internal interpretation. By developing disease risk prediction models, clinicians can rapidly identify high-risk patients from large case datasets and stratify confirmed cases by risk, thereby guiding the formulation of individualized treatment plans.

3. Early-Stage Risk Factors in Children with EBV-Associated HLH

The pathogenesis of EBV-HLH involves a complex interplay of clinical parameters, routine laboratory tests, immunological and inflammatory markers, virological indicators, and genetic factors. Identifying these risk factors is essential for developing and implementing risk prediction models in clinical practice.

3.1. Clinical Indicators

The primary pathophysiological mechanism of EBV-associated HLH in children is aberrant macrophage activation and a fulminant cytokine storm [13], which frequently manifests as cascading multisystem involvement. The early clinical presentation is highly variable and progresses rapidly.

An irregular fever pattern is typically the first and most common warning sign. Nearly all children with EBV-HLH exhibit persistent, irregular fever [3], with patients usually presenting with abrupt-onset, sustained high fever; peak temperatures frequently reach or exceed 39°C [14]. This refractory fever, which shows strong resistance to conventional antimicrobial therapy, directly reflects a sustained, high-intensity state of systemic immune activation driven by the virus.

As the inflammatory cascade intensifies, large numbers of activated lymphoid and phagocytic cells infiltrate the reticuloendothelial system, causing progressive hepatosplenomegaly that accompanies the protracted high fever [15]. Clinically appreciable organ enlargement serves as a visible manifestation of extensive immune cell infiltration and correlates closely with early-stage organ damage.

More concerningly, when this aberrant systemic inflammation breaches the blood-brain barrier, the disease rapidly progresses to central nervous system involvement [16][17]. The emergence of neuropsychiatric manifestations such as irritability, lethargy, and seizures indicates severe brain involvement and constitutes an independent high-risk factor for early mortality in patients with persistent high fever and rapidly progressive organomegaly. Therefore, early clinical evaluation of this condition should include a comprehensive assessment of findings that are frequently mistaken for those of other diseases, rather than simply documenting isolated symptoms.

3.2. Results of Routine Laboratory Tests

3.2.1 Complete Blood Count

Peripheral blood cytological parameters in pediatric EBV-associated HLH include white blood cell count, absolute neutrophil count, red blood cell count, hemoglobin concentration, and platelet count. Central hematopoiesis is profoundly suppressed by a cytokine storm characterized by elevated inflammatory cytokines [18]. Simultaneously, an overactive mononuclear phagocyte system engages in nonspecific phagocytosis of autologous peripheral blood cells.

A marked decline in platelet count is frequently the initial manifestation of this combined effect, which merges central hematopoietic failure with severe peripheral destruction. As immune decompensation worsens, the initial monocyte-specific cytopenia rapidly progresses to refractory progressive cytopenia affecting two or even three cell lineages [1][19]. Severe depletion across multiple cell lineages indicates that the body has exceeded physiological compensatory limits, reflecting complete exhaustion of bone marrow hematopoietic reserves and severe exacerbation of systemic hyperinflammation.

The sharp decline in all blood cell counts accelerates disease progression toward an irreversible critical stage by severely impairing baseline immune barriers and coagulation mechanisms, substantially elevating the risk of fatal infections and secondary massive hemorrhage [4][20]. Incorporating the dynamic trajectory of progressive decline in two- or three-lineage blood cell counts as core feature variables into high-dimensional prediction models enables accurate capture of the microscopic features of early-stage fatal immune activation, providing essential foundational data for optimizing clinical risk classification and establishing a high-precision early warning system.

3.2.2 Liver Function

Liver involvement is the most prevalent and severe visceral manifestation in early-stage EBV-associated HLH in children, as documented by numerous pathological and clinical studies [15]. Dramatic fluctuations in multiple biochemical markers directly reflect severe inflammation and focal necrosis in the hepatic microcirculation.

In the very early phase, a high-concentration cytokine storm and extensive parenchymal infiltration by activated macrophages cause acute tissue injury, manifesting as an abrupt elevation in transaminases [21][22]. Progressive hypoalbuminemia and hyperbilirubinemia follow the collapse of hepatic anabolic compensatory mechanisms, signifying abrupt failure of fundamental physiological processes.

Lactate dehydrogenase (LDH) typically exhibits a rapidly rising trend that correlates closely with worsening liver enzyme abnormalities, serving as a critical indicator of extensive tissue necrosis and highly activated mononuclear phagocytes [23]. This process, marked by a propensity toward acute liver failure, accurately quantifies the degree of immunological injury to the reticuloendothelial system. It provides a crucial quantitative feature variable for constructing high-precision risk prediction models and serves

as an objective benchmark for warning of fatal multiple organ dysfunction syndrome.

3.2.3 Coagulation Parameters

Pathological observations confirm that abnormal activation and depletion of the coagulation system are hallmarks of the aggressive course of EBV-associated HLH in children [19]. This microenvironmental imbalance is directly reflected in dramatic fluctuations of parameters such as fibrinogen, D-dimer, and prothrombin time.

The superimposition of fulminant inflammation and a cytokine storm severely damages endothelial cells, extensively activates the coagulation cascade, and stimulates macrophages to phagocytose platelets and excessively secrete plasminogen activators. These processes drive the body toward a state of disseminated intravascular coagulation characterized by concurrent hyperconsumption and hyperfibrinolysis [18][24]. This homeostatic collapse manifests as significant prolongation of prothrombin time, a sharp rise in D-dimer levels, and a progressive decline in fibrinogen due to the dual impact of massive consumption and hepatic parenchymal failure.

3.2.4 Triglycerides

Disruption of lipid metabolism equilibrium is a fundamental characteristic of EBV-associated HLH in children [3], with the sharp elevation of serum triglycerides representing the most pathologically specific feature. Early in the disease, massive release of pro-inflammatory mediators such as interferon and tumor necrosis factor triggers a robust inflammatory cascade that substantially reduces lipoprotein lipase activity in peripheral capillaries. Triglyceride levels rise sharply due to the combination of severe peripheral clearance impairment and sustained hepatic production [25].

This aberrant elevation is strongly positively correlated with the degree of macrophage activation and infiltration, as well as the intensity of the cytokine storm. Progressive metabolic imbalance, characterized by severe hypertriglyceridemia, accurately measures the intensity of early systemic inflammatory responses and serves as an independent biochemical window for clinically capturing the features of lethal immune activation and assessing the risk of systemic metabolic failure.

3.3. Markers of Inflammation and Immunology

3.3.1 Ferritin

Markedly elevated serum ferritin concentration is the most specific inflammatory marker for assessing EBV-associated HLH in children and is specifically listed as a critical criterion in the HLH-2004 diagnostic guidelines [3]. Immunopathological research and prospective cohort analyses [26][27] indicate that extreme elevation of serum ferritin directly reflects a severe systemic cytokine storm and lethal overactivation of macrophages.

In the early stages of disease, the reticuloendothelial system is continuously stimulated by high concentrations of interferon and pro-inflammatory mediators, leading to pathological proliferation and aberrant hemophagocytosis by tissue mononuclear macrophages. This directly causes activated cells to secrete excessive amounts of ferritin into the peripheral blood [28][29]. The absolute peak and dynamic rate of increase of extreme hyperferritinemia exceed traditional acute-phase reaction thresholds and exhibit an exponential, explosive rise in the very early stages. The degree of uncontrolled immune activation and the risk of multi-organ failure are strongly positively correlated with these ferritin dynamics.

The dynamic trajectory of serum ferritin concentration is a crucial independent biological marker for determining both short- and long-term prognosis in affected children. Large-scale follow-up cohort studies have validated that exceptionally high baseline levels, together with failure to decline or even progressive rebound after targeted therapy, indicate an exceptionally high short-term risk of mortality [27].

3.3.2 Cytokines

The cytokine storm is a key pathogenic feature of pediatric EBV-HLH and persists throughout the disease course. Immunopathological studies have demonstrated that initiation of a lethal cytokine storm constitutes the primary molecular basis for aberrant macrophage activation and multi-organ failure. Recent research has identified aggregation of $IDO1^+$ monocytes and metabolic reprogramming involving L-kynurenine as important immunological characteristics that differentiate EBV-HLH from self-limiting IM [29].

In the early stages of illness, cytotoxic T lymphocytes and natural killer cells targeted by the virus undergo aberrant clonal proliferation, secreting copious quantities of pro-inflammatory cytokines, predominantly TNF-α and interferon-γ (IFN-γ), and establishing a positive-feedback inflammatory loop. This triggers cascade release of secondary inflammatory mediators by activated macrophages, rapidly resulting in severe systemic tissue damage and capillary leakage.

Concurrently, IL-10, an essential component of the body's negative feedback regulatory mechanism, exhibits a compensatory rapid increase in the very early stages. However, rather than effectively halting the inflammatory cascade, its highly abnormal serum concentration, in combination with pro-inflammatory factors such as IFN-γ, creates an immunodysregulatory microenvironment characterized by an imbalance between pro-inflammatory and anti-inflammatory responses. Multiparameter monitoring has proven more accurate than single-factor analysis for identifying high-risk pediatric patients. Several clinical risk stratification models have integrated baseline levels and dynamic clearance rates of IFN-γ and IL-10 [11][30], providing essential information to guide targeted therapies such as emapalumab and stratified interventions. A strong positive correlation exists between significant reduction in these pro-inflammatory cytokine concentrations following targeted immunosuppressive and remission-inducing therapies and substantial extension of overall progression-free survival in pediatric patients.

3.4. Virological Markers

Persistently and abnormally elevated viral copy numbers serve as the direct trigger for lethal overactivation of host macrophages and systemic cytokine storms [31]. In the early disease stages, severe defects or depletion in the capacity of natural killer cells and specific cytotoxic T lymphocytes to clear viral target cells result in uncontrolled viral replication within tissues, leading to detection of extremely high levels of viral nucleic acid in peripheral blood.

This initial high viral load, which breaches the body's primary immune defenses, manifests as an exponential surge in the very early stages; its absolute peak is typically strongly positively correlated with the severity of uncontrolled inflammatory exacerbation and the risk of severe target organ involvement [32]. As systemic immune decompensation worsens, abnormally high levels of viral nucleic acids act as persistent antigenic stimuli, further amplifying and sustaining the inflammatory response and resulting in a trend of persistently high or progressively rebounding viral load on dynamic monitoring. A study employing liquid biopsy technology suggests that analysis of copy number variations in circulating tumor DNA from peripheral blood may assist in diagnosing potential lymphoma when tissue biopsy results are negative, opening new avenues for the differential diagnosis of EBV-HLH [33].

3.5. Genetic Factors

Assessment of potential genetic susceptibility to EBV-associated HLH in children has focused primarily on analysis of pathogenic variants in genes linked to primary immunodeficiencies. Multicenter investigations have identified pathogenic mutations in genes associated with cytotoxic pathways, such as PRF1, UNC13D, and STXBP2, in some children with secondary infection [34]. Under high viral load conditions during the acute disease phase, these variants can rapidly induce lymphocyte degranulation failure and cytokine storm, resulting in explosive, uncontrollable disease progression.

Mutations in the SH2D1A (XLP1 type) and XIAP/BIRC4 (XLP2 type) genes significantly influence the onset and progression of EBV-associated HLH [13][35]. Defects in the signal lymphocyte activation molecule-associated protein encoded by SH2D1A directly disrupt T-cell receptor signaling, causing loss of negative immune regulation in effector cells, which subsequently become persistently overactivated with severely compromised apoptotic clearance mechanisms. Defects in XIAP, a member of the apoptosis-inhibiting protein family, compound impaired autophagy in mononuclear and macrophage systems, promoting release of pro-inflammatory factors such as IL-1β and intensifying the inflammatory response, while simultaneously weakening the capacity of cytotoxic T lymphocytes to specifically eliminate EBV-infected target cells.

Clinical cohorts confirm that EBV readily induces substantial immunological activation in infants with such genetic abnormalities, with the B-cell lineage being the primary target of pathogen invasion. Additionally, the long non-coding RNA LINC02446 is highly expressed in EBV-infected NK cells and promotes HLH development by upregulating IL-10 and KLR family genes. Its expression level correlates positively with ferritin levels and EBV-DNA load [36], making it a potential therapeutic target and prognostic marker.

3.6. Summary of Risk Factors

Table 1 provides a consolidated summary of the principal risk factors discussed above, including their association direction with EBV-HLH, strength of evidence, and inclusion status in existing prediction models.

Table 1 - Summary of Early-Stage Risk Factors for EBV-Associated HLH in Children

Category	Key Indicator	Association Direction	Evidence Strength	Inclusion in Existing Models	Key References
Clinical	Persistent high fever (>39°C)	Positive	Strong	Yes (diagnostic criterion)	[3, 14]
Clinical	Hepatosplenomegaly	Positive	Strong	Yes (clinical presentation)	[15]
Clinical	CNS involvement	Positive	Moderate	Emerging	[16, 17]
Complete Blood Count	Thrombocytopenia	Positive	Strong	Yes (Li model)	[1, 19]
Complete Blood Count	Bicytopenia/pancytopenia	Positive	Strong	Yes (diagnostic criterion)	[4, 20]
Liver Function	Elevated LDH	Positive	Strong	Yes (Li model)	[23]
Liver Function	Elevated transaminases	Positive	Moderate	Partial	[21, 22]
Coagulation	Elevated D-dimer	Positive	Moderate	Yes (Cai model)	[37]
Coagulation	Decreased fibrinogen	Positive	Moderate	Yes (Su model)	[19]
Metabolism	Elevated triglycerides	Positive	Strong	Yes (Cai model)	[3, 25]
Inflammation	Elevated ferritin	Positive	Strong	Yes (Cai model, Su model)	[3, 26, 27]
Inflammation	Elevated IFN-γ, IL-10	Positive	Strong	Yes (Xu model)	[29, 30, 41]
Virological	High EBV-DNA load	Positive	Strong	Partial studies	[31, 32]
Genetic	UNC13D, SH2D1A mutations	Positive	Moderate	Case/family studies only	[13, 34, 35]

4. Predictive Models for Early Diagnosis of EBV-Associated HLH

Early symptoms of EBV-associated HLH in children lack specificity and often manifest as fever, hepatosplenomegaly, and complete blood count abnormalities, showing substantial overlap with clinically common IM and other infectious diseases. Although the HLH-2004 diagnostic criteria serve as the gold standard for HLH diagnosis [3], key indicators such as severe pancytopenia or bicytopenia and extreme hyperferritinemia often do not fully manifest until the middle or late disease stages. This frequently results in delayed treatment. Moreover, because there is no established hierarchy among these diagnostic indicators, the condition is easily confused with critical illnesses such as sepsis, posing significant challenges for early diagnosis of EBV-HLH in children. In recent years, with advances in machine learning algorithms and accumulation of clinical data, researchers have begun exploring the development of predictive models for early diagnosis of EBV-HLH, aiming to provide clinicians with objective and efficient diagnostic tools.

4.1. Models for Early Diagnosis Using Routine Indicators

Predictive models based on common laboratory indicators have emerged as the preferred approach for large-scale screening in early detection of EBV-associated HLH because of their practicality, affordability, and ease of implementation. Early research focused primarily on analyzing associations between individual routine indicators and EBV-HLH.

Hypercytokinemia resulting from aberrant macrophage activation, together with the extrinsic coagulation pathway triggered by interferon-γ, induces hyperfibrinolysis via the interleukin-1β-mediated kallikrein pathway, creating a complex microenvironment of coagulation disorders marked by coexisting hypercoagulability and hyperfibrinolysis. A substantial increase in plasma D-dimer levels and progressive decline in fibrinogen signal this severe imbalance in the coagulation network. Studies have systematically established the considerable clinical utility of markedly elevated D-dimer levels and hypofibrinogenemia for early warning and diagnosis of HLH [37]. By deeply integrating extremely elevated D-dimer levels and characteristic

hypofibrinogenemia as core feature vectors into early-risk prediction models, researchers can significantly enhance the sensitivity of high-dimensional algorithms in capturing nonlinear deterioration trajectories, thereby substantially improving overall diagnostic performance in dynamic differentiation of life-threatening conditions.

Since single indicators cannot fully capture the complex pathophysiological changes associated with EBV-HLH, multiple studies have attempted to develop predictive models integrating multiple indicators. A landmark study published by the team at Hunan Children's Hospital [38] was the first to establish a screening score model for EBV-HLH based on routine laboratory indicators. This study included 3,183 hospitalized pediatric patients with EBV infection. Five independent variables were identified using logistic regression analysis to construct a risk prediction model for early-stage EBV-HLH:

$$\text{Logit}(p) = 9.32 - 0.04 \times \text{Hb} - 0.008 \times \text{PLT} - 0.31 \times \text{NEUT} - 0.195 \times \text{ALB} + 0.002 \times \text{LDH}$$

This model demonstrated a sensitivity of 89.2% and a specificity of 89.5% in distinguishing EBV-HLH from non-HLH cases, with a negative predictive value as high as 99.5%. This extremely high negative predictive value holds substantial clinical importance.

A study by Cai et al. [14] further validated the combined value of multiple routine markers in early identification of EBV-HLH and developed a model:

$$\text{Logit}(P) = -9.969 + 0.057 \times \text{IL-10} + 0.006 \times \text{ferritin} + 0.113 \times \text{D-dimer} + 0.434 \times \text{triglycerides}$$

This model achieved an ROC-AUC value of 0.992, with sensitivity and specificity for early differential diagnosis reaching 97.96% and 98.16%, respectively. These results confirm that a combined predictive model based on cross-system core biochemical indicators demonstrates significant diagnostic efficacy and important clinical translational value in overcoming the limitations of early emergency differential diagnosis and achieving high-precision clinical early warning.

A recent study by Su et al. [39] developed a random forest predictive model based on routine laboratory parameters, further expanding the clinical application of traditional laboratory tests in precise early warning of the disease. Using propensity score matching to balance differences between groups, the study identified four independent risk factors via LASSO regression: hemoglobin (OR = 1.116, 95% CI = 1.050–1.188), ferritin (OR = 0.996, 95% CI = 0.994–0.998), fibrinogen (OR = 7.807, 95% CI = 2.452–24.853), and CD3+CD4+ (OR = 0.805, 95% CI = 0.729–0.889). The model demonstrated a sensitivity of 95.30% and a specificity of 99.70%, indicating good accuracy and predictive capability.

Existing studies confirm that conventional laboratory indicators influencing early onset of EBV-associated HLH in children do not operate in isolation but rather form a complex interactive network through highly correlated pathophysiological mechanisms. Progress in modeling research indicates that risk prediction systems have undergone a paradigm shift from exploration of single indicators to integration of multidimensional features. However, current predictive models are predominantly constructed using single-center retrospective data. Due to limitations in sample size and insufficient geographical representativeness, the generalizability and robustness of existing evaluation systems in cross-regional clinical application continue to face significant challenges.

4.2. Diagnostic Frameworks Combining Immunological Markers and Cytokines

To overcome early differentiation bottlenecks, it is now essential to construct precision diagnostic models that extensively incorporate cytokines and immunological phenotypes, which provide direct reflection of fundamental pathogenic mechanisms. According to state-of-the-art single-cell sequencing studies [40], the core of this illness is a cytokine storm triggered by immunological dysregulation. The microenvironment shows extensive activation of the NF-κB pathway and excessive production by T lymphocytes and natural killer cells. Monocytes significantly differentiate toward an inflammatory phenotype, with aggregation of $IDO1^+$ subpopulations. The specific elevation of their downstream metabolite, L-kynurenine, directly mediates cascading release of pro-inflammatory mediators. This intricate relationship between metabolic reprogramming and systemic inflammatory storm not only reveals the underlying pathogenic drivers but also provides a theoretical foundation for constructing high-precision early-risk prediction models that combine multidimensional cytokines with deep-level immune phenotypes.

Large-scale research has demonstrated the crucial importance of specific cytokine expression patterns in HLH subtype classification and prognostic evaluation [30][41]. Cohort data confirm significant variation in IL-6, IL-10, and IFN-γ levels among different pathogenic subtypes. The multidimensional cytokine dynamic patterns elucidated by this investigation offer a strong theoretical basis for developing high-precision risk prediction models. Using data from pediatric patients, Xu et al. [30] developed a four-quadrant model showing that different HLH subtypes exhibit distinct cytokine patterns. Specifically, the model demonstrated that the IL-10/IFN-γ ratio is a predictive marker for EBV-HLH in children, with an AUC value of 0.877 (95% CI = 0.746–0.897). Additionally, the ROC-AUC value for IFN-γ in predicting EBV-HLH in pediatric patients was 0.723 (95% CI = 0.611–0.836), indicating high discriminatory power.

Abnormal distributions of lymphocyte subsets are crucial for understanding the etiology of severe EBV-associated HLH in children. In contrast to the immunological homeostasis maintained by CD8+ cell proliferation during benign illnesses, the immune systems of children with severe infections become entirely dysregulated and overactivated. Studies indicate that high CD4+/CD8+ ratios constitute an independent risk factor for disease flare-ups (OR = 17.60, 95% CI = 1.89–163.64) [42].

Beyond abnormal expansion of cytotoxic T lymphocytes, collapse of the innate immune clearance barrier and loss of peripheral tolerance are also core mechanisms driving rapid deterioration of EBV-associated HLH in children. Progressive decreases in natural killer cell numbers and significant impairment of perforin production directly prevent the body from eliminating chronically expanding virus-infected cells in high-risk pediatric patients, leading to uncontrollable macrophage activation. Furthermore, a substantial reduction in the percentage of regulatory T cells, which serve as the primary negative regulatory hub maintaining immune homeostasis, further disrupts the peripheral immunological tolerance network. Deeply integrating core quantitative indicators of lymphocyte subsets as key feature variables into high-dimensional predictive model algorithms can greatly improve the ability to identify high-risk individuals at the earliest stages, while also providing vital evidence for optimizing clinical risk stratification and multimodal joint early warning systems.

Cutting-edge multimodal research is overcoming the limitations of single-marker approaches by extensively combining metabolic imaging characteristics with key immunological molecules to create innovative prediction models. Clinical research [43] has demonstrated that high-dimensional fusion of interferon-γ, EBV nucleic acid load, and maximum standard uptake value ratio of lesions can accurately map early pathology and shows remarkable utility in mortality risk stratification. This novel paradigm, which connects microscopic immune activity with macroscopic metabolic imaging, reveals a strong correlation between immune

dysregulation and poor prognosis. This modeling approach, which combines immunology and imaging, is anticipated to significantly advance both cohort management studies of severe cases and early precision warning in children. These indicators provide a crucial molecular foundation for developing high-precision diagnostic models by revealing the essence of the immunopathological features of EBV-HLH from multiple perspectives.

4.3. Models for Molecular Genetic Diagnosis

Molecular genetic etiological diagnostic models are rapidly overcoming the technological limitations of conventional etiological distinction. The Onco-mNGS technique enables simultaneous differentiation of infectious and neoplastic causes by analyzing chromosomal copy number variations (CNVs) and infectious pathogens in a single test. A study by Wu et al. [44] confirmed that high CNV burden, frequent deletions of chromosome 19, and high pathogen burden are significantly associated with poor prognosis. The researchers developed a random forest classification model and a hierarchical diagnostic flowchart combining CNV profiles, infectious pathogen profiles, and peripheral blood microbiome data from Onco-mNGS testing. The ROC-AUC value for high CNV burden was 0.786 (95% CI = 0.651–0.920), whereas the ROC-AUC value based solely on microbiome and clinical data was 0.727, enabling efficient classification of probable causes of secondary HLH.

In contrast, genetic susceptibility screening focuses primarily on primary immunodeficiency disorders and genetic variants associated with familial HLH. Gu et al. [45] reported a patient with compound heterozygous mutation in the UNC13D gene who experienced EBV-HLH. NK cell function tests in family members verified that the UNC13D c.2588G>A single-allele mutation partially reduces NK cell cytotoxicity. Under circumstances such as EBV infection, this variant may function as a genetic risk factor, rendering carriers more susceptible to HLH, lymphoma, and other conditions.

A comprehensive etiological diagnostic system encompassing genetic background, infectious etiology, and tumor-driven factors may be established through synergistic application of the aforementioned molecular diagnostic approaches. Future research should focus on multicenter prospective cohort validation to explore multimodal etiological diagnostic models that thoroughly integrate molecular genetic information with clinical characteristics and imaging measures.

5. Conclusions and Future Perspectives

This article has reviewed research on early risk factors and prediction models for HLH in children with EBV infection. A progressive framework for early identification model development has been delineated, beginning with broad-based screening, advancing to precise identification, and culminating in a molecular genetic perspective. The analysis confirms that combining multidimensional data significantly enhances the performance of predictive models.

Nevertheless, studies on risk factor identification for early-stage EBV-HLH in children vary considerably in terms of disease presentation, patient populations, and variables incorporated into prediction models. This heterogeneity suggests that establishing a standardized, practical screening protocol that accounts for timing of onset, disease severity, and concurrent infections is essential for improving the accuracy and clinical applicability of future predictive models.

Despite considerable progress, several obstacles persist in risk prediction model research. Most current models are derived from single-center or small-scale multicenter studies conducted in China and lack robust external validation. Furthermore, genetic diagnostic markers are absent from the vast majority of early clinical diagnostic protocols due to their high cost and limited clinical accessibility.

Future research should therefore prioritize several directions. First, multicenter collaboration should be strengthened to develop diagnostic models that rely primarily on routine diagnostic procedures supplemented by genetic information. Second, model quality should be systematically evaluated using the PROBAST prediction model assessment tool. Third, advanced machine learning techniques should be introduced to perform dynamic time-series analysis and multimodal data integration, thereby enhancing capacity to capture complex pathological features. Fourth, collaborative efforts should focus on constructing risk prediction models with strong clinical utility and high interpretability.

From early risk detection to targeted intervention, these advances are expected to optimize the entire clinical pathway for pediatric EBV-HLH.

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