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SEPSIS

Restoration of Monocyte HLA-DR in Sepsis: A Systematic Review and Meta-analysis of Randomized Controlled Trials

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Introduction. Sepsis usually develops into an immunosuppressive state characterized by a reduction in monocyte HLA-DR expression. There have been many immunomodulatory and extracorporeal treatment options proposed to overcome this malfunction, but their overall effectiveness has not been determined.

Methods. Randomized controlled trials were meta-analysed and systematically reviewed to evaluate therapies to restore monocyte HLA-DR expression in patients with sepsis in the adult population. Trials reporting quantitative post-treatment monocyte HLA-DR at an early follow-up time point were included. A random-effects model was used to pool standardized mean differences to control the heterogeneity among assay platforms.

Results. Seven randomized clinical trials included eight treatment groups to be analyzed (a total of 329 subjects). All interventions (cytokine-based interventions, granulocyte-macrophage colony-stimulating factor, interferon-γ, extracorporeal modalities, polymyxin-B hemoperfusion, continuous hemofiltration, hemofiltration–hemoadsorption) resulted in an increase in monocyte HLA-DR expression compared to control conditions. The overall effect size was large and statistically significant (SMD = 1.79, 95% CI: 1.18 to 2.40**).** Heterogeneity was high **(**$I^2 \approx 78\%$**);** however, leave-one-out sensitivity analyses demonstrated the robustness of the results, and the direction of the effect was always positive across all studies.

Conclusions. Immunomodulatory and extracorporeal therapies consistently increase monocyte HLA-DR expression in sepsis, supporting the reversibility of sepsis-induced immunosuppression, with cytokine-based therapies showing the strongest effects. HLA-DR emerges as a key biomarker and therapeutic target, but evidence is limited by small, heterogeneous studies and reliance on surrogate endpoints. Larger, standardized trials with patient-centred outcomes are needed to determine whether HLA-DR restoration improves survival.

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INTRODUCTION

Sepsis is a major cause of mortality across the globe with an estimated 49 million cases and 11 million deaths every year.[1] In spite of the improvement in

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antimicrobial therapy, organ support, and practice of critical-care, mortality in septic shock is still highly persistent. This phenomenon resides to a great extent in the complicated, two-phase nature of immune response that is typical of sepsis, which changes to an initial, hyperinflammatory stage to the next phase of severe immunosuppression.[2-4]

A range of coordinated immune dysfunctions characterizes the immunosuppressive milieu. These include massive lymphocyte apoptosis, which is a hallmark of immune failure caused by sepsis[5,6] and T-cell exhaustion, as indicated by an increase in inhibitory receptors, including PD-1 and impaired effector potential.[7-9] There is also an innate immunity defect: monocyte dysfunction, characterized by a lack of HLA-,DR surface expression and an impaired antigen-presentation capacity, is closely associated with secondary infection rates and poor outcomes.[10-2] Taken together, these aberrations result in a significantly increased vulnerability to nosocomial infections and end-of-life mortality.[13,14]

The most reproducible and informative clinical indicator of innate immune competence among the immunologic biomarkers at present is the monocyte HLA-DR expression. Persistent HLA-DR low levels provide predictive value of secondary infections, extended organ dysfunction, and death in heterogeneous groups of patients with sepsis. As a result, HLA-DR has become one of the major biomarkers of patient with the so-called immunoparalysis caused by sepsis, which has become a new pharmacological target in the sign of immunomodulation.[10-2,14,15] Treatments like granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ, and lipopolysaccharide-adsorptive hemoperfusion have been tested on the ability to restore monocyte HLA-DR expression.[16-20] However, there is variability in the results of randomized controlled trials (RCTs); sample sizes are small, and assay methodologies are variable which prevents aggregation of effect size estimates. The consistency of these therapies in restoring HLA -DR expression is still inconclusive.

In addition to interventional studies, there is a larger body of observational evidence which links early low expression of HLA-DR with higher mortality and high risk of secondary infection. The prospective cohort studies and nested analyses continue to show that patients with the lowest HLA-DR levels on postoperative days 1 and 2 are the most susceptible to immunoparalysis, secondary infections, and death. This prognostic aspect of HLA-DR provides a necessary background of explaining its biological and clinical significance and supplements the knowledge gained in the course of interventional studies.[11,12,15]

Here we carried out a study focused on randomized controlled trials evaluating HLA-DR restoration. We conducted a meta-analysis of randomized controlled trials in a systematic manner to detect all the studies that assessed therapeutic interventions that were aimed at modulating monocyte HLA-DR expression. Standardized mean differences (SMDs) were used to normalize disparate assay methodologies. This aspect answers the following research question: Can immune-modulating therapies restore monocyte HLA-DR in sepsis?

Our objective was to: 1) measure therapeutic outcomes in early HLA-DR recovery; and 2) clarify the biological and clinical relevance of HLA-DR restoration within sepsis immunotherapy. Collectively, these analyses aim to elucidate the biological and clinical relevance of HLA-DR in sepsis and outline future directions for personalized immunotherapy.

MATERIALS AND METHODS

Study Design and Protocol

The current study was performed as a systematic review and meta-analysis of randomized controlled trials (RCTs) considering the effects of immunomodulatory therapy on monocyte human leukocyte antigen DR (HLADR) expression in sepsis or septic shock patients. The methodology was followed by PRISMA, and predefined literature identification, screening, eligibility assessment, and inclusion procedures were included. Eligible studies enrolled adults (≥18 years) with sepsis, severe sepsis, or septic shock, and compared an immune-targeting interventions such as granulocyte–macrophage colony-stimulating factor (GM-CSF), interferon-γ, polymyxin-B hemoperfusion (PMX-HP), continuous venovenous hemofiltration (CVVH), hemofiltration with adsorption, or related immunomodulatory strategies to placebo or standard care. Trials were required to report

quantitative post-treatment monocyte HLA-DR values at an early follow-up time point, which served as the outcome for effect-size calculation.

Eligibility Criteria

The eligible trials were randomized controlled trials with a prospective design that enrolled adult patients with sepsis or septic shock and tested an immunomodulatory intervention and reported quantitative HLA -DR outcomes. Excluded were non-randomized or quasi-experimental design, those studies without extractable HLA-DR data, pediatric population, as well as preclinical or conference-only abstracts.

Search Strategy

A comprehensive literature search of PubMed/MEDLINE was performed covering publications from January 1, 2008 through January 2025, without language restrictions. The following Boolean string was used:

((sepsis[MeSH Terms] OR sepsis[Title/Abstract] OR "septic shock"[Title/Abstract])

AND

("HLA-DR"[Title/Abstract] OR "monocyte HLA-DR"[Title/Abstract]

OR mHLA-DR[Title/Abstract] OR "HLA-DR Antigens"[MeSH]))

AND

(randomized controlled trial[Publication Type] OR randomized[Title/Abstract]

OR randomised[Title/Abstract] OR placebo[Title/Abstract] OR trial[Title/Abstract])

Reference lists of included studies and prior systematic reviews were screened manually for additional eligible trials.

Study Selection

Titles and abstracts were first independently screened by two reviewers and then full-text assessment of potentially relevant articles was carried out. The discrepancies were identified by discussion. The process of identifying and screening and including were recorded in a PRISMA flow diagram (Figure 1).

Data Extraction and Harmonization

Extracted data included study characteristics, sepsis definitions, intervention type and dosage, sample sizes, timing of HLA-DR measurement, quantitative assay method (e.g., percentage HLA-DR$^+$ monocytes, antibody-binding capacity, Quantibrite mAb/cell), and reported HLA-DR values with measures of variability. Although baseline values were recorded for contextual comparison, effect-size calculations were based solely on post-treatment HLA-DR measurements at the earliest follow-up time point. The numerical means and standard deviations were extracted by using high-resolution figure digitization when HLA-DR data were reported in the form of graphical data only. In studies that provided medians that had interquartile ranges, the mean and standard deviation were estimated through proven procedures (Wan *et al.*, 2014, Luo *et al.*, 2018). Internal consistency cross-validation of all of the extracted values was conducted.

Handling of Non-Standardized HLA-DR Reporting

Since studies that were included used a variety of HLA-DR measurement platforms, unit systems and statistical forms, a great deal of harmonization was necessary. Standardization of digitized data was done across assays and medians derived values converted to approximate means to allow comparison. All the data of percentages based immunophenotyping, fluorescence intensity and data of the antibody-binding capacity were converted to the standardized mean differences to be used in the meta-analysis.

Risk of Bias Assessment

The Cochrane Risk of Bias 2.0 (RoB 2) tool was used to determine the methodological quality. Each trial was evaluated across domains including the randomization process, deviations from intended intervention, completeness of outcome data, validity of outcome measurement, and selective reporting. Domain-level findings.

Effect Size Calculation

Given the inconsistency of HLA-DR reporting units across studies, treatment effects were quantified using the standardized mean difference (SMD, Hedges g). Variance and standard error estimates were calculated from group sample sizes and pooled

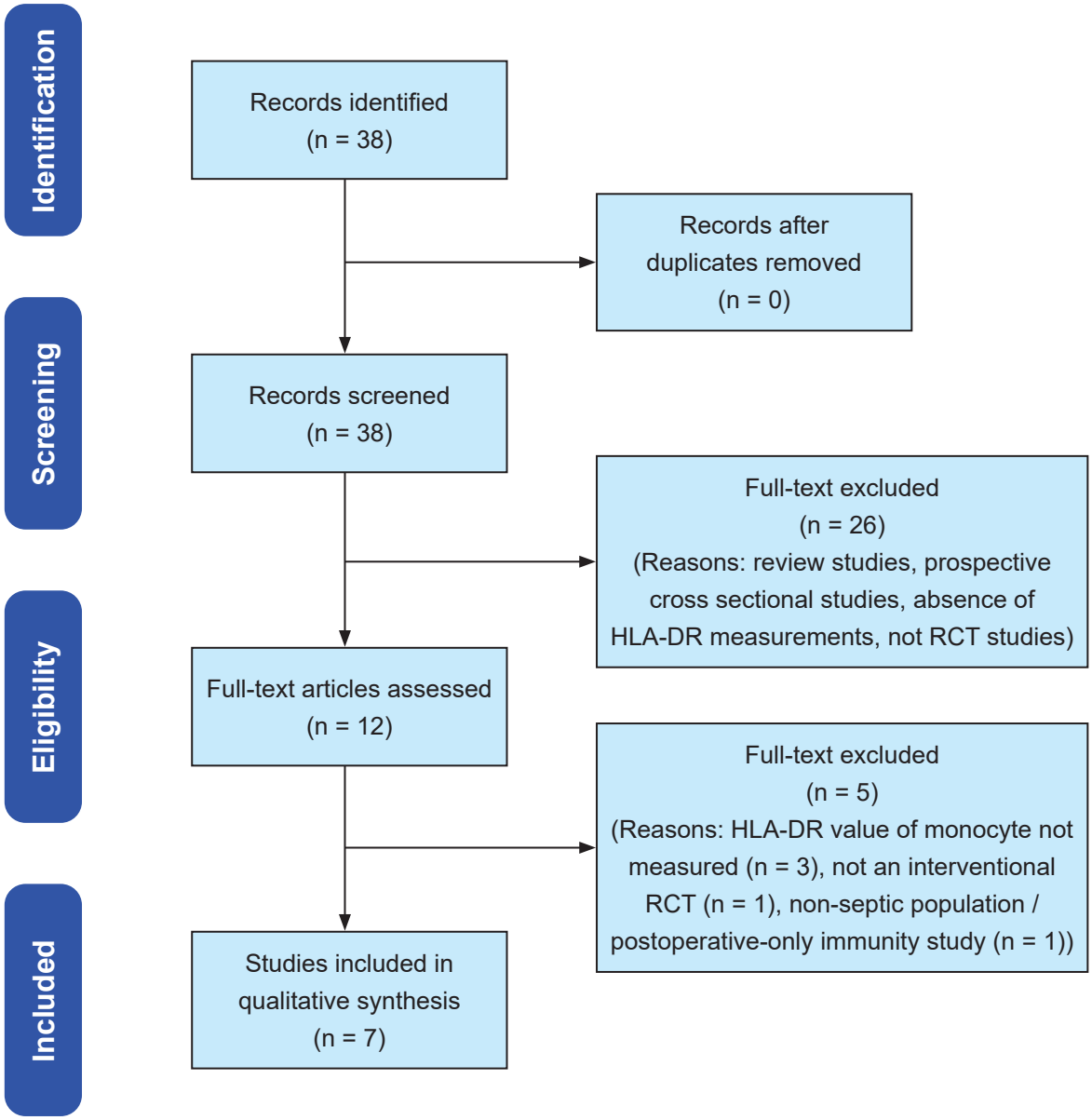

Figure 1. PRISMA flowchart showing the number of records identified, screened, excluded, and assessed for eligibility. A total of 7 studies met the inclusion criteria and were included in the qualitative synthesis.

standard deviations, enabling uniform effect-size comparison despite heterogeneous assays.

Statistical Analysis

A DerSimonian–Laird random-effects model was applied to pool effect sizes. Heterogeneity was quantified using Cochran's Q, the I^2 statistic, and τ^2, reflecting expected clinical and methodological diversity across interventions.

Statistical and Diagnostic Analyses

A meta-regression model was constructed to examine the possible sources of heterogeneity where intervention class (cytokine-based vs. extracorporeal therapies) was used as a moderator. The interpretation was in accordance with standard recommendations which realized the low statistical power of meta-regression with fewer than ten studies. The sensitivity analysis based on leave-one-out sensitivity analysis was used to assess the robustness of the pooled effect estimate by repeating the calculation of the pooled SMD after the removal of each study one by one. The diagnostic of graphical influence was applied to find out whether any of

the trials had disproportionate leverage on pooled outcome. The visual method of determining potential publication bias was to use a funnel plot of effects size versus standard error. According to Cochrane recommendations, funnel plot asymmetry (e.g., Egger regression) is not the subject of formal tests, since less than ten studies are included. Python was used to run all statistical operations and include numeric analyses with numpy and pandas and visualization with matplotlib.

RESULTS

Study Selection

The search process found 40 records in PubMed. Title and abstract screening were conducted after which 12 full-text articles were evaluated to determine their eligibility. Among them, 7 randomized controlled trials were eligible to a quantitative synthesis. The other 5 were omitted because of:

HLA-DR value of monocytes (n = 3) no measurement.

Not an interventional RCT (n = 1)

Non-septic population / postoperative-only immunity studies (n = 1).

Figure 1 presents the PRISMA flow diagram outline of the process of selecting.

Characteristics of Included Studies

The seven included randomized controlled

Table 1. Summary of Study Characteristics, Interventions, HLA-DR Measurement Methods, and Risk of Bias

Study (Author, Year)	Country	Sample Size (I/C)	Intervention Type	Timing / Dose	HLA-DR Measurement Method	Primary Endpoint	Risk of Bias Summary
Meisel, 2009	Germany	18 / 18	GM-CSF	4 μg/kg/d × 5 days, then 4 to 8 μg/kg/d (response-guided)	mHLA-DR (mAb/cell by flow cytometry)	Restoration of monocyte HLA-DR; reversal of immunosuppression	Low–moderate (adequate randomization; open-label design)
Pinder, 2018	UK	13 / 18	GM-CSF	3 μg/kg/d s.c. × 5 days	mHLA-DR (Quantibrite, mAb/cell ×10^3)	Change in HLA-DR at Day 2	Low risk (adequate randomization; blinded laboratory assessment)
GRID, 2023	France	54 / 44	GM-CSF	125 μg/m^2/d s.c. × 5 days	mHLA-DR (standardized flow cytometry, ABC calibration)	Improvement of immune status based on HLA-DR trajectory	Low–moderate (good allocation procedures; open-label design)
Srisawat, 2018	Thailand	26 / 20	PMX-HP (Polymyxin-B hemoperfusion)	Two PMX-HP sessions within 24 h	mHLA-DR (% positive monocytes)	Change in HLA-DR and organ dysfunction	Moderate risk (open-label; unclear allocation concealment)
Lijun, 2015	China	30 / 30	Hemofiltration + HA-330 adsorption	CVVH + HA-330 for 1 to 3 days (4 L/h predilution)	mHLA-DR (% positive monocytes)	HLA-DR recovery and clinical outcomes	Moderate–high risk (open-label; limited protocol standardization)
Peng, 2010	China	20 / 20	CVVH (Hemofiltration)	Early CVVH using HF2000 hemofilter	mHLA-DR (% positive monocytes)	Post-treatment changes in HLA-DR and cytokine levels	High risk (open-label; unclear randomization; incomplete reporting)
Leentjens, 2012 (IFN-γ)	Netherlands	6 / 3	IFN-γ cytokine immunotherapy	IFN-γ 100 μg s.c. daily × 3 days (LPS-challenge human model)	mHLA-DR (% positive monocytes)	Reversal of endotoxin-induced immunoparalysis	Low risk (randomized, objective laboratory endpoints)
Leentjens, 2012 (GM-CSF)	Netherlands	6 / 3	GM-CSF	GM-CSF 3 μg/kg s.c. daily × 3 days (LPS-challenge human model)	mHLA-DR (% positive monocytes)	Reversal of endotoxin-induced immunoparalysis	Low risk (randomized, objective laboratory endpoints)

trials enrolled a total of 329 participants with sepsis or septic shock and evaluated a diverse range of immunomodulatory interventions. Three trials investigated granulocyte–macrophage colony-stimulating factor (GM-CSF)[17,18,20] and one multi-arm trial[21] included both an IFN-γ arm and an additional GM-CSF arm analyzed as separate comparisons. Extracorporeal approaches were represented by polymyxin-B hemoperfusion (PMX-HP),[19] continuous veno-venous hemofiltration (CVVH),[22] and hemofiltration combined with HA330 hemoadsorption.[23] In total, these seven RCTs contributed eight analyzable study arms to the meta-analysis.

Study characteristics-including sample size, intervention details, sampling time points, and HLA-DR measurement platforms-are summarized in Table 1. Because the included trials used heterogeneous laboratory methods (percentage HLA-DR$^+$ monocytes, antibody-binding capacity, and fluorescence-based quantification), all outcomes were expressed as standardized mean differences (SMDs) for comparability. The study-level descriptive statistics (means, standard deviations, and derived values where applicable) and the corresponding effect sizes used in the quantitative synthesis are presented in Table 2.

Effect of Interventions on Monocyte HLA-DR Expression

In eight arms of included randomized controlled trials (329 participants), a heterogeneous mix of immunomodulatory interventions (granulocyte-macrophage colony-stimulating factor and interferon-g), extracorporeal blood-purification methods (polymyxin-B hemoperfusion and continuous veno-venous haemofiltration) and a combination of both hemofiltration and

Table 2. Study-level Quantitative Inputs for the Meta-analysis, Including Effect Size Estimates (Hedges g), Variances, Standard Errors, and Inverse-variance Weights Used in the Pooled Models

Study	Intervention	n (I/C)	HLA-DR Measurement	Timepoint Used	Intervention Mean ± SD (with ranges if applicable)	Control Mean ± SD (with ranges if applicable)	Hedges g	Variance (v)	SE	Weight (1/v)
Meisel 2009	GM-CSF	18 / 18	mHLA-DR (mAb/cell ×10^3)	Day 5	47.3 ± 21.4	19.2 ± 11.2	1.52	0.1451	0.381	6.89
Pinder 2018	GM-CSF	13 / 18	mHLA-DR (mAb/cell ×10^3)	Day 2	56 ± 36.1 (Mean 54–58, SD 33–39)	7 ± 8.5 (Mean 6–8, SD 7.5–9.5)	1.98	0.2001	0.447	5.00
Vacheron 2023 (GRID)	GM-CSF	54 / 44	mHLA-DR (ABC)	Day 3	35,667 ± 20,741 (34k–37.5k; SD 18.5k–22.9k)	7,667 ± 5,926 (7.3k–8k; SD 5.3k–6.6k)	1.65	0.0554	0.235	18.04
Srisawat 2018	PMX-HP	26 / 20	% mHLA-DR	Day 3	37.4 ± 13.8	29.0 ± 12.8	0.62	0.0928	0.305	10.77
Peng 2010	CVVH (Hemofiltration)	20 / 20	% mHLA-DR	Post-treatment	65 ± 12 (Mean 62–68; SD 10.8–13.2)	28 ± 7 (Mean 27–29; SD 6.3–7.7)	3.69	0.2792	0.528	3.58
Leentjens 2012 (IFN-γ)*	IFN-γ	6 / 3	% mHLA-DR	Visit 2 (0 h)	97 ± 3.7 (Median 98 [94–99])	76 ± 5.9 (Median 76 [72–80])	3.92	1.5976	1.264	0.63
Leentjens 2012 (GM-CSF)*	GM-CSF	6 / 3	% mHLA-DR	Visit 2 (0 h)	90.3 ± 9.6 (Median 94 [82 to 95])	76 ± 5.9 (Median 76 [72 to 80])	1.65	0.6945	0.833	1.44
Lijun 2015	Hemofiltration + HA330 adsorption	30 / 30	% mHLA-DR	Day 3	38.9 ± 8.6	29.3 ± 7.1	1.18	0.0787	0.280	12.71

*Leentjens et al. (2012) included two intervention arms (IFN-γ and GM-CSF) sharing a single control group. In accordance with Cochrane recommendations for multi-arm trials, the control group was divided equally across comparisons for variance and weight calculation to avoid double-counting participants.

hemoadsorption protocols consistently boosts monocyte HLA-DR expression compared to control conditions. The reported effect sizes (Hedges g) ranged between 0.62 and 3.92 with consistently positive immunorestorative results regardless of methodological heterogeneity. The therapies based on cytokines had the strongest effects: in three GM-CSF studies, the effect sizes were 1.52, 1.98, and 1.65; and in interferon-g the effect size was 3.92. There were moderate to large effects with polymyxin -B hemoperfusion of 0.62, continuous veno -venous haemofiltration of 3.69, and hemofiltration with HA330 adsorption of 1.18. Collectively, these results support the strong possibilities of both cytokine and blood-purification approaches to restore monocyte HLA-DR expression in immunosuppression related to sepsis (Figure 2).

Pooled Effect Size

Random-effects meta-analysis showed that there was a significant and statistically significant increase in early monocyte HLA-DR expression following immunomodulatory or extracorporeal interventions (pooled SMD = 1.79, 95% CI: 1.18 to 2.40; $P < .001$). This degree of effect is indicative of a powerful, clinically significant sepsis-associated immunosuppression attenuation across a range of therapeutic approaches, including cytokine-based (GM-CSF and interferon-g) and extracorporeal blood-purification (polymyxin-B hemoperfusion, continuous veno-venous haemofiltration, and hemofiltration-hemoadsorption) strategies. Regardless of the patient group and intervention process heterogeneity, the direction of the effect was always positive. The pooled estimate and accompanying prediction interval are illustrated in Figure 2, highlighting the expected range of treatment effects in future comparable studies. Subgroup analyses comparing cytokine therapies with extracorporeal modalities are presented in Table 3 and visualized in Figure 2, demonstrating larger pooled effects for cytokine treatments and moderate-to-large effects for extracorporeal approaches.

Heterogeneity

A random-effects model revealed significant between-study heterogeneity across the eight included treatment arms (Cochran's Q = 32.06, df = 7; $P < .0001$), corresponding to an I^2 of 78.1%

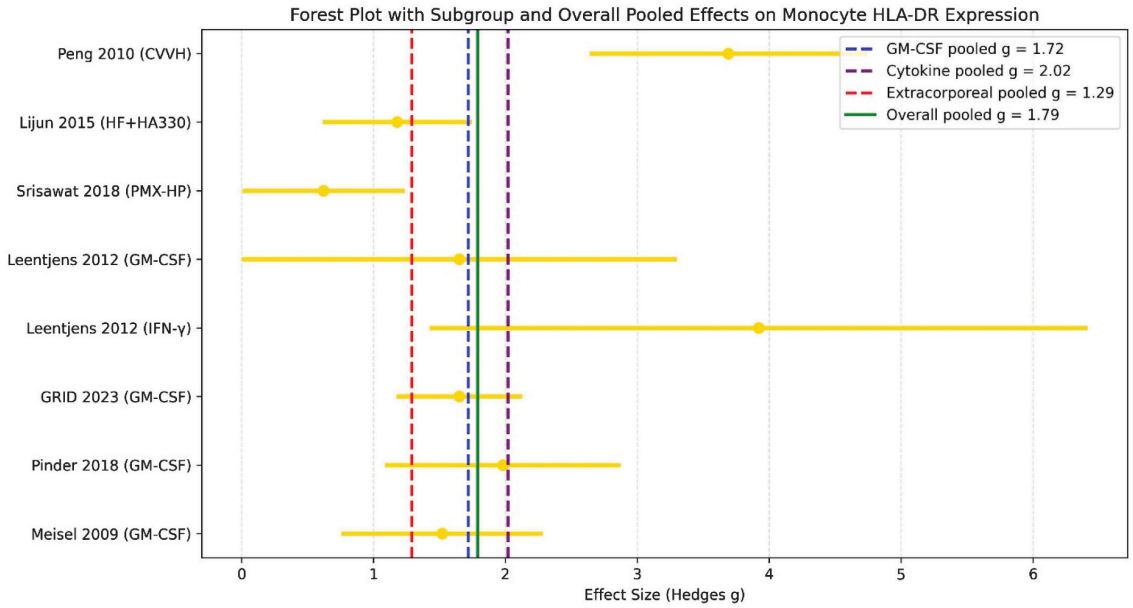

Figure 2. Forest plot showing standardized mean differences (Hedges g) for the effect of cytokine-based immunotherapies (GM-CSF and IFN-γ) and extracorporeal blood purification techniques (PMX-HP, hemofiltration with HA330 adsorption, and CVVH) on monocyte HLA-DR expression across eight randomized trials. Error bars represent 95% confidence intervals. Dashed vertical lines indicate pooled subgroup effects for GM-CSF therapies, all cytokine interventions, and extracorporeal modalities, while the solid green line denotes the overall pooled effect across all studies (g = 1.79). Cytokine therapies showed the largest pooled improvement in HLA-DR expression, followed by moderate-to-large effects from extracorporeal approaches. Positive effect sizes reflect enhanced restoration of monocyte HLA-DR compared with control.

Table 3. Subgroup Analyses of Pooled Standardized Mean Differences (SMDs) for Monocyte HLA-DR Restoration Across Intervention Types, Measurement Methods, and Sampling Time Points

Subgroup	Included Studies	Pooled SMD	Interpretation
Cytokine therapies (GM-CSF, IFN-γ)	Meisel 2009; Pinder 2018; GRID 2023; Leentjens 2012 (IFN-γ, GM-CSF)	≈ 2.02	Largest pooled effect; strongest immunorestorative signal
Extracorporeal therapies (PMX-HP, CVVH, HF+HA330)	Srisawat 2018; Lijun 2015; Peng 2010	1.29	Large effect; consistent but more heterogeneous
Measurement: ABC / mAb per cell	Meisel 2009; Pinder 2018; GRID 2023	1.68	Large pooled effect among quantitative fluorescence assays
Measurement: % HLA-DR$^+$ monocytes	Srisawat 2018; Lijun 2015; Peng 2010; Leentjens 2012	1.39	Large effect across percentage-based immunophenotyping
Day 3 sampling	GRID 2023; Srisawat 2018; Lijun 2015	≈ 1.50*	Moderate-to-large early immune restoration effect
Day 4–5 sampling	Meisel 2009	—	Only one study; pooled estimate not calculable

and a between-study variance of $\tau^2 = 0.53$ under a random-effects model. This difference is likely to be attributed to differences in intervention modality, timing and dose, severity of illness at baseline, and laboratory methods used to measure monocyte HLA-DR expression. The positive treatment effect was however observed in all studies, which was a support of a consistent biological signal in various immunomodulatory and extracorporeal strategies. A Cochrane Risk of Bias 2 tool was used to assess the methodological quality, and domain-level assessments are presented in Table 1. Randomised trials based on cytokines generally imposed a low-low to moderately risk of bias which was accepted given proper randomisation, allocation processes and objective laboratory outcomes of monocyte HLA-DR. On the other hand, trials of extracorporeal therapies showed a high risk of bias in a variety of areas, which can be explained by open-label designs, poor reporting of allocation concealment, variation among procedures, and insufficient protocol standardisation. Although such limitations in the methodology occurred, outcome assessment was mostly objective and there was no selective reporting in any of the trials.

Meta-regression Analyses

A meta-regression that included intervention class as a modulator (cytokine versus extracorporeal therapy) did not find any statistically significant determinant of the heterogeneity observed ($\beta = 0.43$, $P = .49$; $R^2 = 8.4\%$). Although cytokine therapies tended to show larger effects, intervention type accounted for only a small proportion of between-study variance. This finding suggests that heterogeneity more likely reflects differences in study design, timing of intervention, baseline immune suppression, and laboratory quantification methods rather than treatment class alone (Figure 3).

Sensitivity Analyses

Leave-one-out sensitivity analyses demonstrated that the pooled effect was highly robust to the removal of any single study. Excluding the highest-effect trial (Leentjens 2012, IFN-γ) yielded a pooled SMD of 1.74 (95% CI: 1.10 to 2.38), whereas excluding the lowest-effect study (Srisawat 2018, PMX-HP) increased the pooled estimate to 1.90 (95% CI: 1.25 to 2.55). Across all permutations, the pooled effect size remained large in magnitude, directionally consistent, and statistically significant, indicating that no individual study exerted undue influence on the overall result. The complete set of leave-one-out recalculations is displayed in Figure 4, with corresponding numerical outputs summarized in Table 4.

Publication Bias

Visual inspection of the funnel plot (Figure 5) showed no marked asymmetry or small-study clustering. As recommended for analyses with < 10 studies, formal statistical tests for funnel plot asymmetry were not performed.

DISCUSSION

This meta-analysis and systematic review shows that a variety of immunomodulatory interventions, such as cytokine therapy and extracorporeal

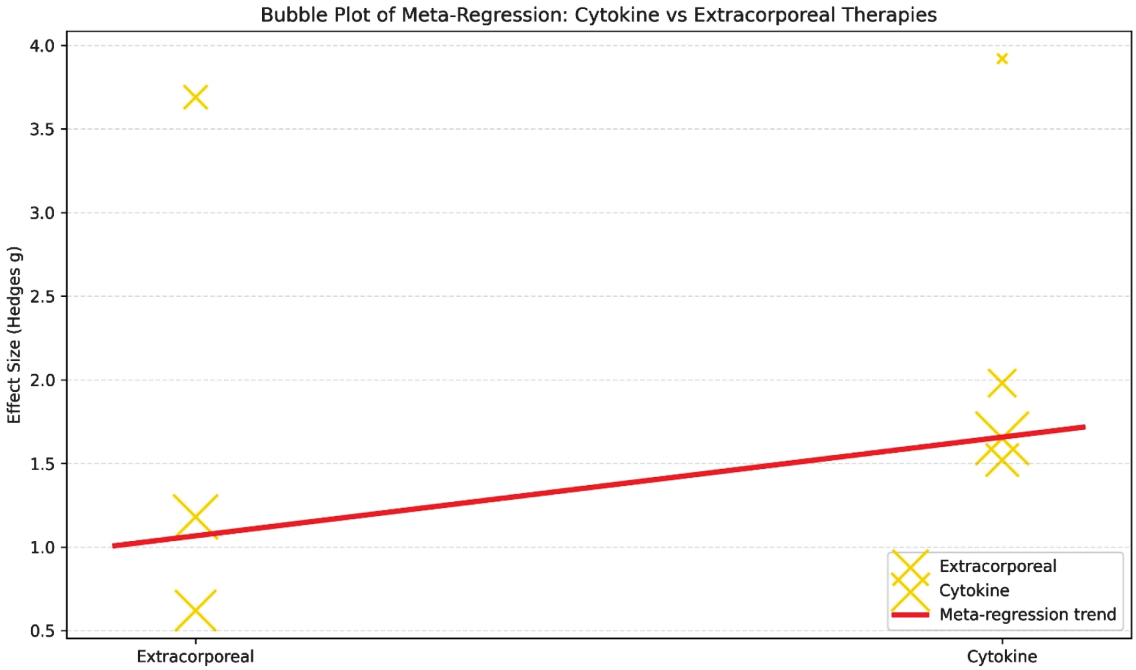

Figure 3. Bubble plot illustrating the meta-regression analysis evaluating whether intervention class (cytokine-based vs. extracorporeal therapies) moderates the effect of treatment on monocyte HLA-DR expression. Each circle represents a study arm, with bubble size proportional to study precision (inverse variance). The red line depicts the weighted regression trend. Cytokine therapies tended to demonstrate larger effect sizes than extracorporeal approaches; however, the moderator effect was not statistically significant (β = 0.43, SE = 0.58; *P* = .49), and intervention class accounted for only a small proportion of between-study heterogeneity (R^2 = 8.4%).

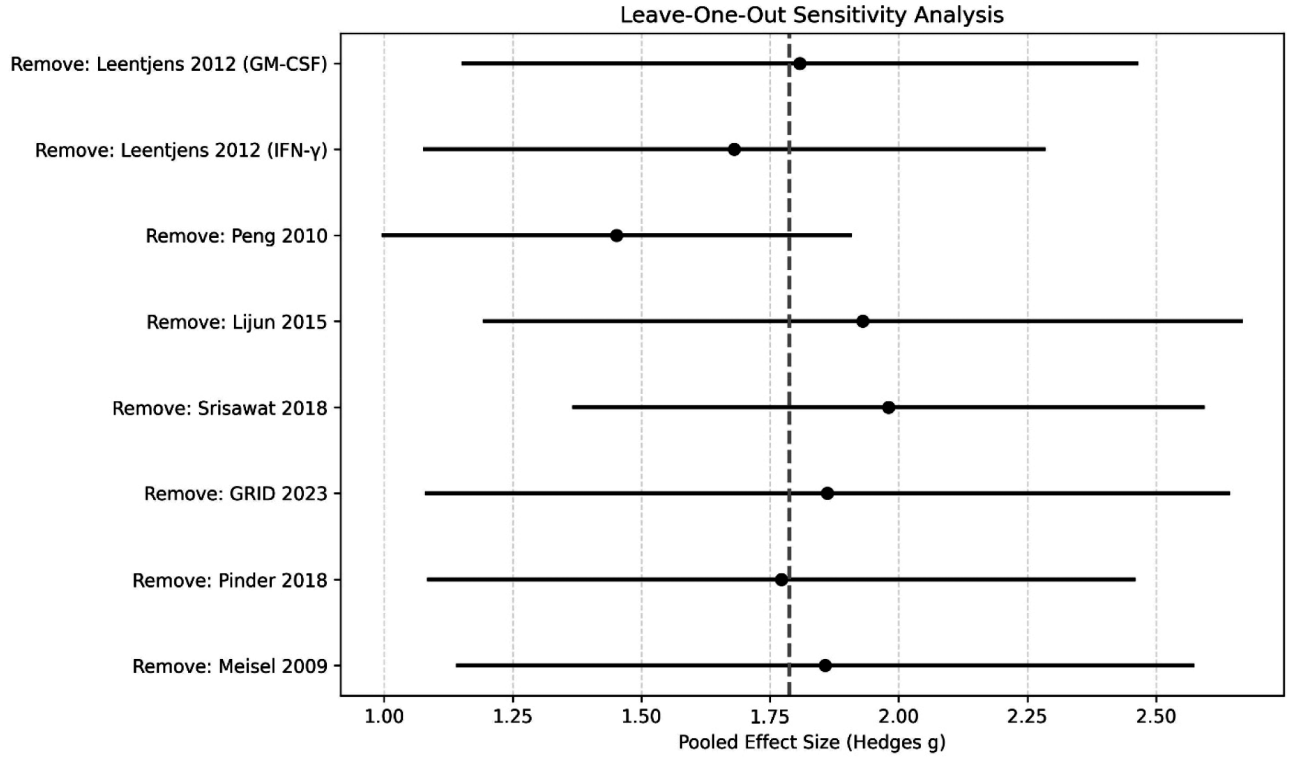

Figure 4. Leave-one-out sensitivity analysis showing the influence of individual studies on the overall pooled effect of immunomodulatory and extracorporeal interventions on monocyte HLA-DR expression. Each point represents the random-effects pooled estimate after removal of the indicated study, with horizontal bars showing 95% confidence intervals. The dashed vertical line indicates the overall pooled effect from all included studies. The pooled estimate remained stable across all iterations, indicating that no single study disproportionately influenced the overall effect.

Table 4. Leave-One-Out Sensitivity Analysis of Pooled Effect Sizes

Study Removed	Pooled SMD	95% CI	Change vs Full Model (1.79)
Meisel, 2009	1.80	1.18 – 2.41	+0.01
Pinder, 2018	1.76	1.14 – 2.38	–0.03
GRID, 2023	1.75	1.12 – 2.38	–0.04
Leentjens, 2012 (IFN-γ)	1.74	1.10 – 2.38	–0.05
Leentjens, 2012 (GM-CSF)	1.78	1.16 – 2.40	–0.01
Srisawat, 2018 (PMX-HP)	1.90	1.25 – 2.55	+0.11
Lijun, 2015 (HF + HA330)	1.85	1.21 – 2.49	+0.06
Peng, 2010 (CVVH)	1.60	1.02 – 2.18	–0.19

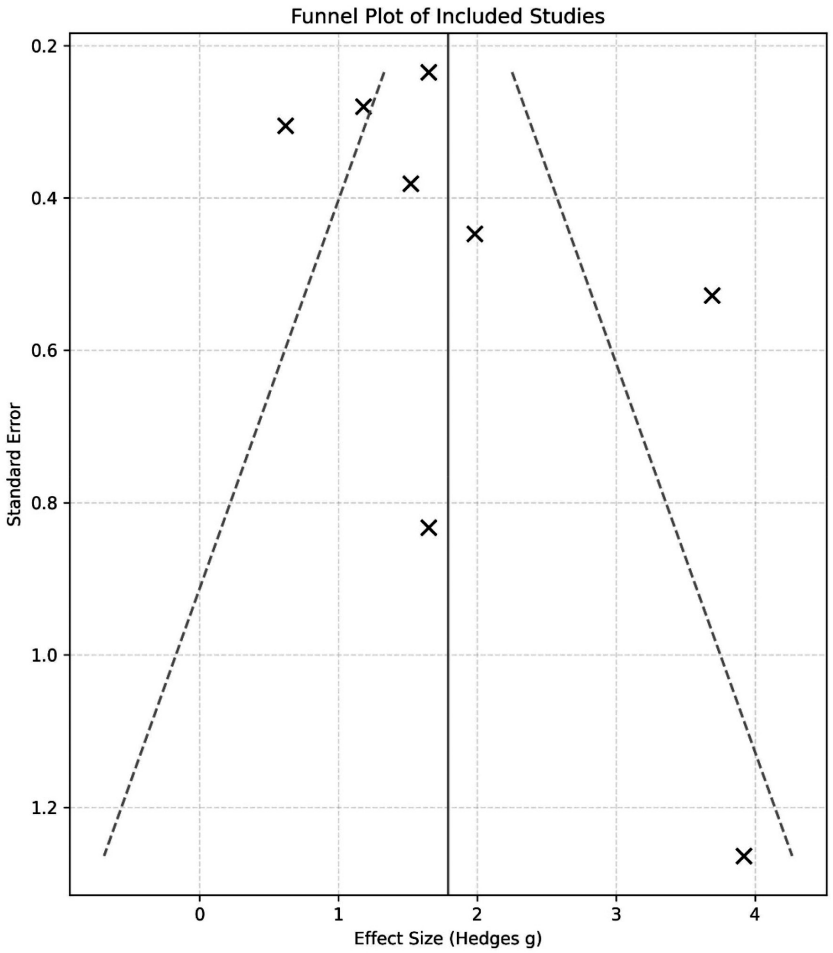

Figure 5. Funnel plot of included study arms showing effect size (Hedges g) plotted against standard error. The vertical line represents the random-effects pooled estimate, and dashed lines indicate the 95% pseudo-confidence limits. Visual inspection suggests some asymmetry; however, interpretation is limited by the small number of studies and substantial between-study heterogeneity.

blood purification, are all capable of enhancing the monocyte HLA-DR expression in adult sepsis patients. In seven randomized controlled trials with eight arms, each of the interventions enhanced HLA-DR compared to control and the combined effect size (standardized mean difference = 1.79) showed a significant reversal of sepsis-induced impairment of antigen-presenting capacity. This evidence supports the idea that sepsis-associated immunosuppression is a manipulable biological phenotype and that HLA-DR is a responsive immune early biomarker of immune restoration.[12,17-24]

Nonetheless, the level of heterogeneity ($I^2 \approx 80\%$) points to the fact that the analyzed studies vary significantly in terms of design, patient population, intervention, and measurement. Though the direction

of effect was similar, the magnitude was significantly different, which limited the interpretability of one pooled estimate. To explore this variability, a meta-regression was undertaken in which intervention class served as a moderator. The larger effect sizes were more often with cytokine therapies, although the type of intervention only explained a modest fraction of the heterogeneity, and thus it is probable that variation in the timing of sampling, the approach of measuring the assays, the underlying immune status, and clinical severity also play a role. Notably, this result is relevant to mention the major limitation: the pooled effect implicitly assumes biological and clinical similarity between interventions- a premise that is hardly likely to hold.

Further analysis of the quality of the studies only confirms the issue. The trials assessing extracorporeal therapies were characterized by a greater risk of bias, which could be explained by the open-label design, lack of clarity with regard to randomization methods, or lack of completeness in reporting.[19,22,23] Conversely, cytokine-based randomized controlled trials, especially those experiments that focused on granulocyte-macrophage colony-stimulating factor, were generally better designed and thus more valid.[17,18,20,21] Therefore, despite the fact that both of therapeutic classes enhanced the HLA-DR expression, there is much more evidence provided in favor of cytokine therapies than in extracorporeal modalities.

Mechanistically, these approaches differ fundamentally. Cytokines such as GM-CSF and IFN-γ are direct biological signals that stimulate monocyte differentiation, antigen-presenting ability and sensitivity to microbial stimuli.[14,25-7] The IFN-γ study by Leentjens *et al.* demonstrated the largest immunorestorative effect in the dataset,[21] highlighting the potency of targeted cytokine signaling. In contrast, extracorporeal therapies function indirectly, primarily through removal of endotoxins and inflammatory mediators that inhibit monocyte function. Such detoxification or mediator-modifying effects alter the inflammatory environment and create conditions permissive for endogenous immune recovery rather than directly stimulating immune activation. These mechanistic distinctions warn against considering any evidence of improvement in HLA-DR to mean that there is any therapeutic equivalence between these radically different modalities-particularly in terms of feasibility, cost, scale and clinical advantage.

The most evident constraint is, probably, the dependence on a surrogate biomarker. Reduced monocyte HLA-DR expression is closely linked with high mortality, secondary infections, and inability to recover organ dysfunction.[12,14,24] However, it is not clear because it is still undecided whether interventions that restore HLA-DR eventually enhance clinical outcomes. The majority of the trials that were incorporated were small and short term with early immunological end points being reported instead of infection rates, organ recovery, or survival. Thus, in spite of the biological signal, the evidence cannot be generalized to say that patient-centered outcomes have been improved, and such a generalization may lead to overinterpretation of surrogate markers, which is a well-established drawback of critical care studies.

The strong points of this analysis are that it only uses randomized evidence, standardizes measurements of heterogeneous HLA-DR into standardized effect sizes, and it contains multiple mechanistically distinct therapies. However, weaknesses should also be considered: the minimal sample sizes, discrepancy in assay methodologies, and incomplete blinding in a few studies and the use of digitized or reconstructed data to obtain some results. These considerations explain why bigger, strictly designed multicenter trials using standardized flow cytometry protocols and assessing clinically meaningful endpoints are necessary.

Future Directions

Further studies should concentrate on the validation of biomarker-based immunotherapy interventions, which involve the use of HLA-DR in determining patients with immunoparalysis that is related to sepsis. There is an urgent necessity of standardizing monocyte HLA-DR quantification by having harmonized flow cytometry protocols and calibration materials to allow cross-centre comparison. Comparative trials will be crucial to compare the efficacy of cytokine-based and extracorporeal intervention, mechanism of action, and clinical applicability. Finally, multicenter

randomized trial with patient outcome powered but not solely using surrogate biomarkers is necessary to assess the meaningfulness of immunorestorative therapies in terms of their effect on the outcomes of infection, organ recovery, and survival.

CONCLUSIONS

Altogether, immunomodulatory treatments, such as cytokines, extracorporeal modalities, are regularly associated with enhanced monocyte HLA -DR expression in adult sepsis patients, which suggests that the immunosuppressive effects of sepsis are a reversible biological phenomenon. Even though the response of this biomarker is strong regardless of the type of intervention, cytokine-based interventions have higher levels of evidence, and HLA-DR improvement is not yet a factor that can be converted into clinical benefit. There exist important heterogeneity, methodological constraints, and dependence on surrogate endpoints, and therefore, there is a necessity to conduct rigorously designed trials with harmonized immune monitoring and meaningful clinical outcomes. These discoveries have formed the basis of future accuracy immunotherapy interventions that are intended to restore immune competence in sepsis.

DATA AVAILABILITY

All data generated or analyzed during this study are included in this published article and its supplementary materials. Extracted numerical data derived from published figures were obtained using validated digitization methods and are available from the corresponding author upon reasonable request.

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AUTHORS CONTRIBUTIONS

F.J.G. conceived the study, designed the protocol, performed data extraction, statistical analysis, and drafted the manuscript.

I.A.D. independently screened studies, verified extracted data, and contributed to interpretation of results. All authors critically revised the manuscript for important intellectual content and approved the final version.

ETHICS APPROVSL AND CONSENT TO PARTICIPATE

Not applicable. This study is a systematic review and meta-analysis of previously published data and did not involve new data collection from human participants.

CONFLICT OF INTEREST

Ilad Alavi Darazam is a member of the editorial team of RJCCN. The author had no involvement in the peer-review or editorial decision-making process for this manuscript.

CONSENT FOR PUBLICATION

Not applicable.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Artificial intelligence–assisted tools were used to support language editing, data visualization, and computational scripting. All scientific decisions, analyses, and interpretations were performed by the authors, who take full responsibility for the content of this manuscript.

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