ORIGINAL ARTICLE

Human Leukocyte Antigen – DR (HLA-DR) Isotype Expression on Monocytes as a Prognostic Marker in Patients with Sepsis in Intensive Care Unit

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ABSTRACT

Background: *Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection. Sepsis begins with excessive inflammatory state caused by pro-inflammatory cytokines which can be followed by Immunosuppression state. During the immune paralysis stage, the level of Human Leukocyte antigen -DR (HLA-DR) expression on monocytes is decreased which increase the risk of secondary infection.* ***Objective:*** *This study aims to evaluate the relation between the level of Human Leukocyte Antigen DR expressed on monocytes (mHLA-DR) and the clinical outcome of septic patients to use mHLA-DR as a prognostic marker for Sepsis.* ***Methodology:*** *Blood samples were withdrawn from 40 patients diagnosed as sepsis in Intensive Care Units in El Demerdash Hospital and 20 apparently healthy individuals. Flowcytometric analysis was used to measure the level of HLA-DR expressed on monocytes. A second sample was taken from each patient after 1 week. For long hospitalized patients, a third sample was taken after 1 more week to follow up the level of mHLA-DR.* ***Results:*** *Percentage of mHLA-DR in the initial sample, First and second follow up samples (median=76.5%) showed highly significant difference compared to the sample taken from control group (median=99.1%). No significant difference was found in mHLA-DR percentage between initial sample (median=76.5%), first follow up sample (median=76.55%), second follow up sample (median=55.6%). There was no statistically significant difference in the percentage of mHLA-DR in the three samples between died and alive patients.* ***Conclusion:*** *The level of mHLA-DR can be used as a predictive biomarker for the clinical outcome of patients with sepsis.*

INTRODUCTION

According to the World health organization (WHO): Sepsis is a severe organ dysfunction due to dysregulated host response to infection [1]. It occurs mainly due to bacterial infection, but viral infections also can lead to sepsis. [2]

The pathogenesis of sepsis depends mainly on the host immune response. Any infection stimulates the immune system to elicit an inflammatory response. If dysregulated inflammation occurs, it will result in sepsis which comprise two phases: the first hyperinflammatory state **(Cytokine storm)** followed by a second phase of immune suppression **(immune paralysis).** [3]

During the Immune paralysis stage, the adaptive immune tolerance counteracts the initial inflammatory state[4]. This stage occurs due to the release of anti-inflammatory mediators as transforming growth factor-β (TGF-β) and IL-10 [5].

Human leukocyte antigen-DR isotype (HLA-DR) expressed on monocytes is a major histocompatibility complex (MHC) class II which is a surface glycoprotein that presents the pathogen to CD4 T-lymphocytes which then respond to the antigen. [6,7] HLA-DR is one of three polymorphic genes of MHC class II [8].

The defect in monocytes leads to low human leukocyte antigen-DR isotype (HLA-DR) surface expression and reduced antigen presentation capacity [9]. The decrease in HLA-DR reflects monocyte dysfunction. So, the decrease in Human Leukocyte Antigen-DR isotype surface expression on monocytes increase the susceptibility of acquiring secondary infection especially opportunistic infections [10]. HLA-DR expression can be an early biomarker of immune suppression as its rule is an early step in the immune response against pathogens [11].

During immunosuppression, death may occur due to acquiring an opportunistic infection, or the reactivation of potential viruses [12]. So, the level of Human Leukocyte Antigen-DR (HLA-DR) expression on monocytes can be a good indicator of the immune suppression state in sepsis and a guide for management.

Aim of work

This study aims to evaluate the relation between the change in the level of Human Leukocyte Antigen DR

expressed on monocytes and the clinical outcome of septic patients to use Human Leukocyte Antigen (HLA-DR) as a prognostic marker for sepsis.

METHODOLOGY

Ethical approval:

The Research Ethical Committee, Faculty of Medicine Ain Shams University, granted approval for this study under the code No: FWA 000017585. Informed consent to participate was obtained from all the patients or their legal guardians.

Settings and study design

This cohort study was carried on over a period of 8 months from November 2022 till June 2023. It was conducted on 40 hospitalized patients in Intensive Care Units (ICU) in different Medical and Surgical Departments in El Demerdash Hospital. All patients were diagnosed with sepsis according to the Third definition of Sepsis by the Society of Critical Care Medicine and the European Society of Intensive Care Medicine [13] which define Sepsis as a life-threatening organ dysfunction caused by a dysregulated host response to infection, where organ dysfunction is identified by acute change in SOFA score by ≥2 points. And 20 non hospitalized apparently healthy individuals were enrolled in the study as a control group.

Exclusion criteria for patients: Patients diagnosed with Human immunodeficiency virus (HIV), Infectious Hepatitis or Autoimmune diseases.

For all patients: Full History was taken, Laboratory investigations result as Complete blood count (CBC), C-reactive protein (CRP), Blood culture, Sequential Organ Failure Assessment (SOFA) score were collected from patients' files.

Methods

Two blood samples (8ml each) were collected from 2 different body sites with at least 1 hour apart under complete aseptic conditions. Each blood sample was added immediately to the blood culture bottle (70 ml); Hi-Safe blood culturing system (Himedia, India).

Another 1 ml blood sample was collected from all patients and controls in an EDTA-treated CBC tube and was used to measure Human Leukocytic Antigen-DR (HLA-DR) level by Flowcytometry. One more blood sample was collected after 1 week. In long hospitalized patients, a third blood sample was taken after 1 week from the first follow up sample.

Blood culture:

Blood culture was kept in the incubator at 37 ♌C. Then subculture was done on suitable media (Blood agar medium, MacConkey's agar medium, Sabouraud's dextrose agar medium) (Oxoid UK). After 24 hours of incubation, Bacterial colonies were identified by culture characteristics, Gram-staining and biochemical reactions (Catalase production test, Coagulase production test, Oxidase production test, Triple Sugar iron test, indole production test, urease production test and citrate utilization test) (Oxoid UK) **[14].**

Flow Cytometric Analysis:

Separation of peripheral blood mononuclear cells from the blood was done using density gradient centrifugation; then purification of the isolated cells was performed using anti-human CD45-PC5 (Beckman Coulter, USA), anti-human CD14-PE (Beckman Coulter, USA), anti-human HLA-DR-FITC (Beckman Coulter, France).

The labeled samples were analyzed using flow cytometry and the NAVIOS CXP software (Beckman Coulter, USA).

Analysis of the results:

Monocytes were gated according to the morphological characteristics of the cells detected by the light scatter parameters. Size of the cells was estimated by forward scatter while Side scatter reflects the internal structure of the cell **[15].**

RESULTS

The study was conducted on 40 patients: 22 females (55%) and 18 males (45%) who were admitted to the Intensive Care Unit (ICU) in El Demerdash Hospital, all patients had Sepsis at the time of study. The study was conducted between November 2022 to June 2023. The age of the patients ranged between 18 to 86 years old. In the healthy control group, there were 16 female (80%) and 4 males (20%), and their age ranged between 21 to 57 years old.

There was no statistically significant difference found between control group and patients group regarding neither sex distribution with p-value = 0.058 nor age. (**Table 1)**

Table 1: Comparison between control group and patients group regarding demographic data

		Control group	Patients group	Test-value	P-value	Sig.
		No.=20	No.=40			
Age	**Mean±SD**	49.60 ± 18.79	58.38 ± 16.88	-1.828•	0.073	NS
	Range	21 – 57	18 – 86			
Sex	**Female**	16 (80.0%)	22 (55.0%)	3.589*	0.058	NS
	Male	4 (20.0%)	18 (45.0%)			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)

*: Chi-square test; •: Independent t-test

CRP level was elevated in all patients with median 153.85 mg/L. SOFA score was calculated for each patient with median of 4 points. The results of blood culture were negative in 25 patients (62.5%). While it was positive in 15 patients (37.5%), the commonly isolated organisms were *Klebsiella pneumoniae*, *Acinetobacter*, *Coagulase-negative staphylococci* (CoNS). (**Table 2)**

Table 2: CRP, SOFA score and blood culture of the studied patients

		Patients group
		No.=40
CRP	**Median (IQR)**	153.85 (97.7 – 223.5)
	Range	22 – 373.2
SOFA	**Median (IQR)**	4 (2.5 – 6)
	Range	1 – 9
Blood culture	**No Growth**	25 (62.5%)
	Klebsiella pneumoniae	2 (5.0%)
	Acinetobacter	3 (7.5%)
	***Coagulase-negative staphylococci* (CoNS)**	3 (7.5%)
	Methicillin-resistant Staphylococcus aureus	1 (2.5%)
	Staphylococcus haemolyticus	1 (2.5%)
	No growth then *Klebsiella pneumoniae*	1 (2.5%)
	Staphylococcus auricularis	1 (2.5%)
	Staphylococcus hominis	1 (2.5%)
	***Klebsiella pneumoniae* (MDR)**	1 (2.5%)
	Staphylococcus epidermidis* then *Diphtheroids	1 (2.5%)

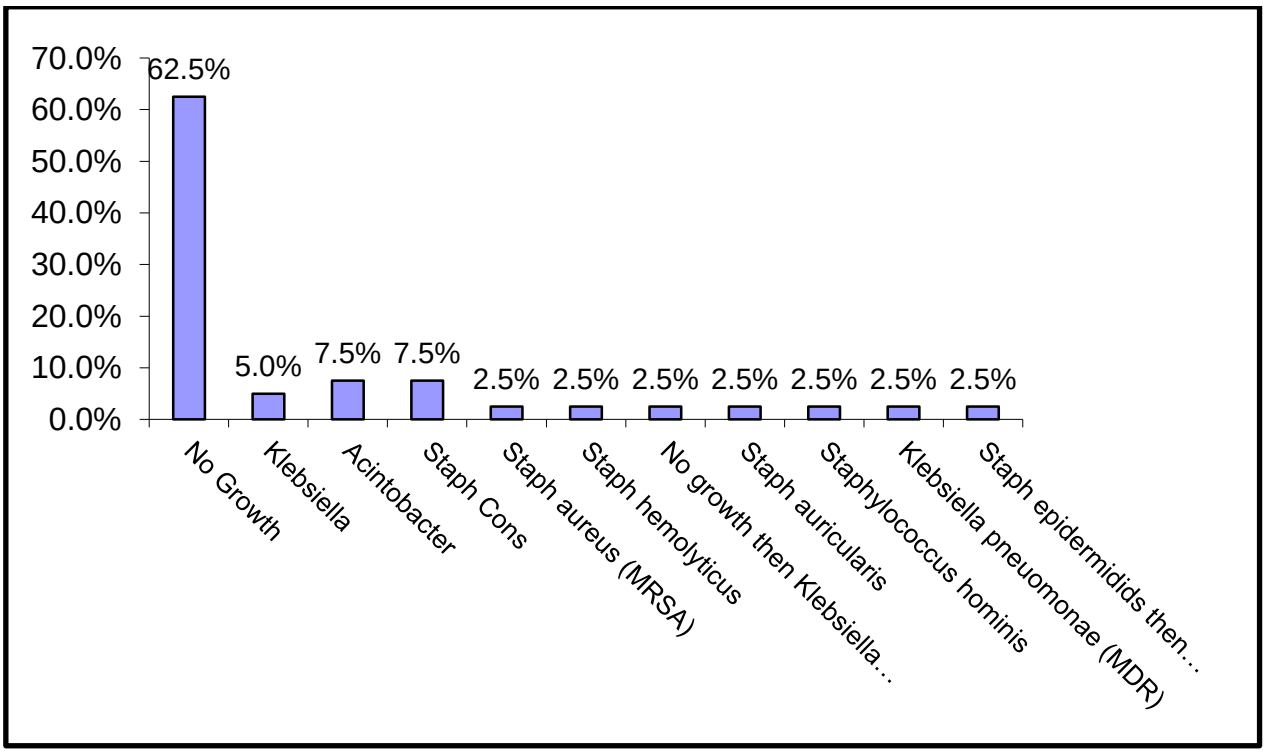

Fig. 1: Results of blood culture

TLC from the initial blood sample in patients (with median=15.95) showed Highly significant difference compared to the healthy control group (with median= 6.5). Percentage of mHLA-DR in the initial sample taken from patients (with median=76.5%) showed highly significant difference compared to healthy control group (with median=99.1%). **(Table 3)**

Table 3:Comparison between control group and patients group regarding first sample of TLC and HLA-DR (%)

		Control group No.=20	Patients group No.=40	Test-value‡	P-value	Sig.
TLC	**Median(IQR)**	6.5 (5.25 - 6.9)	15.95 (10.3 – 23.35)	-5.803	0.000	HS
	Range	3.23 – 6.9	5.6 – 45.9			
HLA-DR%	**Median(IQR)**	99.1 (97.85 – 99.95)	76.5 (57.05 – 91.1)	-5.702	0.000	HS
	Range	90.9 – 100	7.2 – 99.1			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Mann Whitney test

TLC from the first follow up sample was higher in patients (with median = 14) than in healthy control group (with median= 6.5). The percentage of HLA-DR on monocytes in the first follow up sample was still lower in patients group (with median=76.55) than in healthy control group (with median= 99.1). **(Table 4)**

Table 4: Comparison between control group and patients group regarding first follow up of TLC and HLA-DR(%)

		Control group No.=20	Patients group No.=40	Test-value‡	P-value	Sig.
TLC 2	**Median (IQR)**	6.5 (5.25 - 6.9)	14 (11.05 - 16.95)	-5.106	0.000	HS
	Range	3.23 – 8.9	4.1 – 45.5			
HLA-DR%	**Median (IQR)**	99.1 (97.85 – 99.95)	76.55 (60.95 – 89.15)	-5.663	0.000	HS
	Range	90.9 – 100	11.5 – 99.2			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Mann Whitney test

TLC was higher in the second follow up sample from long-hospitalized patients (with median=14.1) than in control group (with median=6.5). The percentage of mHLA-DR was significantly lower in the second follow up sample from long hospitalized patients (with median=55.6) than in healthy control group (with median= 99.1). **(Table 5)**

Table 5: Comparison between control group and patients group regarding second follow up of TLC and HLA-DR (%)

		Control group No.=20	Patients group No.=8	Test-value‡	P-value	Sig.
TLC 3	**Median (IQR)**	6.5 (5.25 - 6.9)	14.1 (9.5 - 17.75)	-3.412	0.001	HS
	Range	3.23 – 8.9	5.7 – 33.4			
HLA-DR%	**Median (IQR)**	99.1 (97.85 – 99.95)	55.6 (10.35 - 81.85)	-4.030	0.000	HS
	Range	90.9 – 100	0 – 91.6			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Mann Whitney test

There was no significant difference in TLC between initial sample, first and second follow up. No significant difference was found in mHLA-DR percentage between initial sample (with median=76.5%), first follow up sample (with median=76.55%), second follow up sample (with median=55.6%). **(Table 6)**

Table 6: Follow up of TLC and HLA-DR (%) of the studied patients

		First sample No.=40	First follow up No.=40	Second follow up No.=8	Test-value‡	P-value	Sig.
TLC	**Median (IQR)**	8.9 (15.95 - 10.3)	14 (11.05 - 16.95)	14.1 (9.5 - 17.75)	1.750	0.417	NS
	Range	5.6 – 45.9	4.1 – 45.5	5.7 – 33.4			
HLA-DR%	**Median (IQR)**	76.5 (57.05 – 91.1)	76.55(60.95–89.15)	55.6(10.35 - 81.85)	1.000	0.607	NS
	Range	7.2 – 99.1	11.5 – 99.2	0 – 91.6			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Fried Mann test

The sensitivity of TLC in the initial sample (TLC 1) was 92.5%, in the first follow up sample (TLC2) it was 80% and 87.5% in the second follow up sample (TLC3). The specificity of TLC in the initial sample was (TLC1), first follow up sample (TLC2) and second follow up sample (TLC3) were 95%, 100%, 95% respectively. **(Table 7)**

The sensitivity of the percentage of mHLA-DR from the initial sample (HLA-DR1) was 87.5% and 80% in the first follow up sample (HLA-DR2).In the second follow up sample (HLA-DR3), the sensitivity was HLA-DR was 100%. The specificity of the percentage of HLA-DR in the initial sample, first follow up and second follow up samples were 90%, 100%, 95% respectively. **(Table 7)**

The cut off point for the percentage of mHLA-DR in the initial sample was ≤ 95.1%. In the first follow up sample it was ≤ 89.8%, and 91.6% in the second follow up sample. **(Table 7)**

Table 7: ROC curve of TLC1 and HLA-DR 1(%) as a predictor of patients.

Parameter	Cut off Point	AUC	Sensitivity	Specificity	PPV	NPV
TLC 1	**>7.99**	**0.962**	**92.5**	**95.0**	**97.4**	**86.4**
HLA-DR (%) 1	**≤95.1**	**0.954**	**87.5**	**90.0**	**94.6**	**78.3**
TLC 2	**>8.9**	**0.907**	**80.0**	**100.0**	**100.0**	**71.4**
HLA-DR (%) 2	**≤89.8**	**0.951**	**80.0**	**100.0**	**100.0**	**71.4**
TLC3	**>7.99**	**0.919**	**87.5**	**95.0**	**87.5**	**95.0**
HLA-DR (%) 3	**≤91.6**	**0.994**	**100.0**	**95.0**	**88.9**	**100.0**

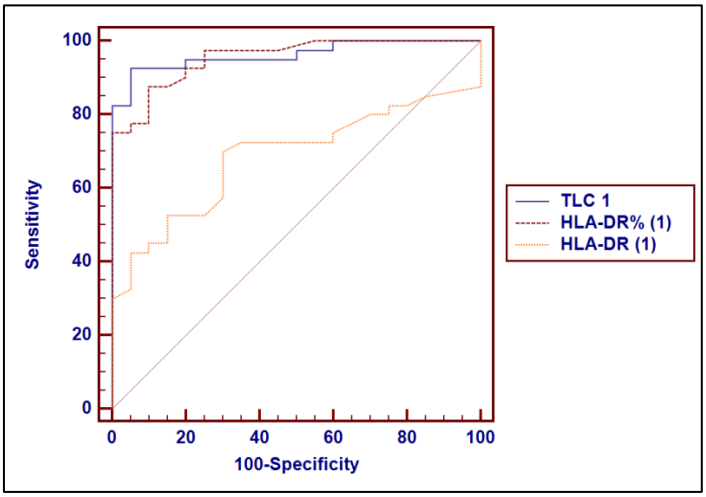

Fig. 2: ROC curve of TLC1 and HLA-DR 1(%) as a predictor of patients.

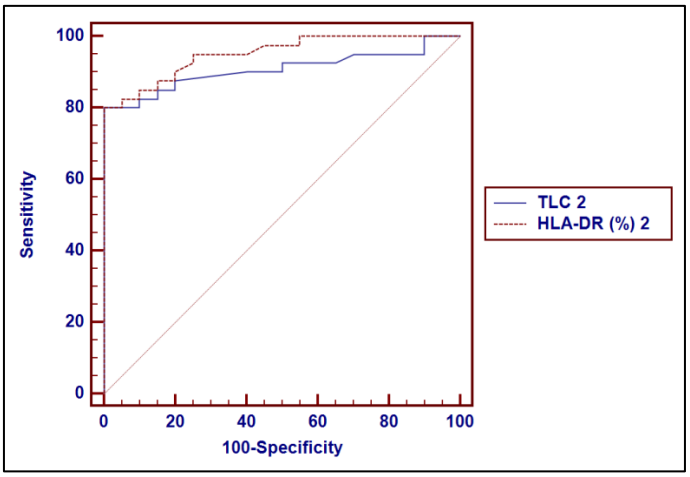

Fig. 3: ROC curve of TLC2 and HLA-DR (%) 2 as a predictor of patients

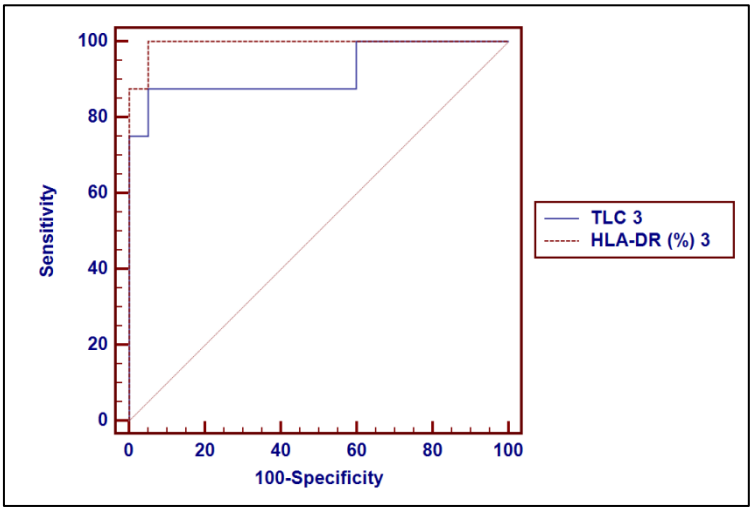

Fig. 4: ROC curve of TLC3 and HLA-DR (%) 3 as a predictor of patients

There was no significant difference between the two groups regarding TLC and mHLA-DR (percentage) in the three samples taken from all patients except TLC in the first follow up sample which is significantly higher in died patients than in alive patients (P-value = 0.015). **(Table 8)**

Table 8: Comparison between alive and died patients regarding first sample, first follow up and second follow up of the studied parameters.

		Alive	Died	Test value‡	P-value	Sig.
		No. = 19	No. = 21			
TLC 1	Median (IQR)	14 (9.3 – 21)	19 (10.6 – 24.6)	-0.989	0.323	NS
	Range	6.5 – 45.9	5.6 – 31.5			
HLA-DR%	Median (IQR)	79 (56.5 – 94.7)	73.4 (57.6 – 88.1)	-0.880	0.379	NS
	Range	23.9 – 99.1	7.2 – 97			
TLC 2	Median (IQR)	11.9 (7.4 – 14.3)	16.1 (13.6 – 19)	-2.438	0.015	S
	Range	4.4 – 33	4.1 – 45.5			
HLA-DR%	Median (IQR)	79.3 (67.4 – 92.4)	73.8 (41.5 – 88.8)	-0.907	0.364	NS
	Range	22.4 – 99.2	11.5 – 97.6			
TLC 3	Median (IQR)	8.2 (5.7 – 17.5)	15.2 (13 – 18)	-1.342	0.180	NS
	Range	5.7 – 17.5	10.8 – 33.4			
HLA-DR%	Median (IQR)	85.9 (71.4 – 91.6)	17.3 (3.4 – 39.8)	-1.938	0.053	NS
	Range	71.4 – 91.6	0 – 77.8			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS)
‡: Mann Whitney test

DISCUSSION

Sepsis remains a critical condition characterized by a dysregulated immune response to infection, contributing significantly to morbidity and mortality worldwide [13]. Human Leukocyte Antigen – DR (HLA-DR) expression on monocytes has emerged as a promising biomarker of sepsis, reflecting the immune status and prognosis of septic patients [16].

HLA-DR molecules play a pivotal role in antigen presentation and immune response modulation. During sepsis, downregulation of HLA-DR on monocytes is associated with immune dysfunction, impaired bacterial clearance, and increased susceptibility to secondary infections [(10)].

This study investigated the potential of Human Leukocyte Antigen – DR (HLA-DR) isotype expression on monocytes as a prognostic marker in patients with sepsis admitted to the Intensive Care Unit (ICU) of El Demerdash Hospital.

Regarding age, the mean age in the patients group (58.38 ± 16.88 years) was slightly higher than that in the control group (49.60 ± 18.79 years). Although this difference was not statistically significant (p = 0.073), it

is consistent with previous studies that have reported old age as a risk factor for severe sepsis and poor outcomes [17]. However, variations in age distribution across studies underscore the complexity of age as a predictor in sepsis outcomes, requiring further investigation in larger, multi-center cohorts.

In terms of sex distribution, our study observed a higher proportion of females in the control group (80.0%) compared to the patients group (55.0%), though this difference did not reach statistical significance (p = 0.058). This finding contrasts with some studies that have reported a higher incidence of sepsis and higher mortality rate among males [18]. The variability in sex distribution across studies may reflect differences in study populations, geographic regions, and underlying comorbidities affecting susceptibility to sepsis [18]. These findings suggest that age and sex, despite being important demographic factors, did not confound the comparison of HLA-DR expression between septic patients and healthy controls in our study.

As regards key clinical parameters including C-reactive protein (CRP) levels, Sequential Organ Failure Assessment (SOFA) scores, and blood culture findings among ICU patients diagnosed with sepsis. CRP levels were uniformly elevated in all patients, with a median of 153.85 mg/L (IQR 97.7 - 223.5), indicating robust systemic inflammation. This aligns with Anush et al. [19] study which reported CRP as a valuable biomarker for monitoring sepsis severity and response to treatment [19].

SOFA scores, reflecting the extent of organ dysfunction, showed a median of 4 points (IQR 2.5 - 6) in our cohort. These results are consistent with other studies which found that Higher SOFA scores are associated with increased mortality and prolonged ICU stays, highlighting the critical role of early assessment and intervention in sepsis management [13, 20].

Blood cultures yielded positive results in 15 patients (37.5%). This is consistent with the results of ***Gonçalves-Pereira***[21] study in 2013 in which 19.9 % of the patient only have positive blood culture. According to the meta-analysis of 10 studies, there was no significant difference in the all-cause mortality and length of ICU stay between positive and negative blood culture patients [22].

Concerning the compared key immunological parameters between patients with sepsis and a healthy control group, focusing on TLC and HLA-DR expression on monocytes as a percentage (%) (Table 4). Regarding Total Leukocytic Count (TLC), The median TLC in patients with sepsis was significantly higher compared to the healthy control group (p = 0.000, highly significant). Hotchkiss study found that Elevated TLC in sepsis reflects a systemic inflammatory response characterized by leukocytosis, indicative of immune activation and response to infection [16].

As regards percentage of Monocytic HLA-DR (%) in this cohort study, Patients with sepsis exhibited a significantly lower median percentage of monocytic HLA-DR expression compared to the healthy control group (p = 0.000, highly significant). Landelle et al. [23] has reported that Decreased HLA-DR expression on monocytes is a well-established marker of immune suppression in sepsis, associated with increased susceptibility to secondary infections and poorer outcomes.

For Total Leukocytic Count (TLC) during the first follow-up in our study, the median TLC in patients remained significantly elevated compared to the healthy control group (p = 0.000, highly significant). This aligns with Hotshkiss who concluded that persistent leukocytosis underscores the ongoing inflammatory response and immune activation in sepsis, despite clinical management and treatment efforts [16].

Regarding percentage of Monocytic HLA-DR (%) in the first follow up sample in our study, Similar to initial measurements, patients with sepsis demonstrated a significantly lower median percentage of monocytic HLA-DR expression compared to healthy controls (p = 0.000, highly significant). This finding agrees with Landelle et al.[23] study which explained that the sustained decrease in HLA-DR expression highlights persistent immune suppression, which can predispose patients to secondary infections and worsen clinical outcomes.

In our study, concerning the second follow up sample taken from long hospitalized patients, Total leukocytic count (TLC) remained significantly elevated in patients compared to controls (p = 0.001, highly significant), consistent with prolonged systemic inflammation and immune activation observed in sepsis, which is the same results obtained from Jang et al. study[24]. This finding underscores the ongoing physiological stress and immune response in critically ill patients despite extended hospitalization and treatment.

In relation to HLA-DR expression on monocytes as a percentage in the second follow up sample in this study, it remained significantly lower in patients compared to controls. This finding agrees with the explanation of Leijte et al.[25] that sustained decrease reflects persistent immune suppression, a critical factor contributing to increased susceptibility to secondary infections and prolonged hospital stays. The variability observed within the patient group highlights the complexity of immune dysregulation in sepsis, influenced by disease severity and individual patient responses.

On the same line Jang et al. [24] supports our finding of elevated TLC in patients with sepsis, indicating ongoing systemic inflammation and immune activation. Also, Leijte et al. [25] and Monneret and Venet [26] agree with our study regarding the significant decrease in HLA-DR expression on monocytes in patients with

sepsis which highlights prolonged immune suppression associated with poor outcomes.

The ROC curves indicate high predictive ability for both TLC and HLA-DR 1 (%) in distinguishing between patient groups based on their respective cutoff points.

Like ROC Curve 1, both TLC2 and HLA-DR 2 (%) show strong predictive performance with high sensitivity and specificity, indicating their potential utility as prognostic markers. TLC3 maintains good sensitivity and specificity, although slightly lower compared to TLC1 and TLC2. HLA-DR 3 (%) shows exceptional performance with a very high AUC (0.994) and perfect sensitivity in identifying patients at risk.

In relation to overall Utility of TLC and HLA-DR (%), The ROC curves demonstrate that TLC and HLA-DR (%) are effective in predicting sepsis outcomes, with varying degrees of sensitivity and specificity across different cutoff points and follow-up periods.

Liu et al**.** [10] was showing consistent findings across different patient populations and settings, affirming the robustness of TLC and HLA-DR (%) in predicting clinical outcomes. Also Leijte et al. [25] found that change in mHLA-DR expression over time is important in the prediction of clinical outcome of septic patients. On the other hand, Perry et al. [27] concluded that low HLA-DR is not associated with high mortality rates.

Agreed studies support the use of these biomarkers, highlighting their reliability and clinical significance in sepsis management. Disagreed studies underscore potential challenges and variability in predictive accuracy, emphasizing the need for further validation and context-specific application.

In our study, the comparison between alive and deceased patients revealed no significant difference in TLC and mHLA-DR percentage across the three samples, except for TLC in the first follow-up sample, which was significantly higher in deceased patients (P-value = 0.015). Jang et al.[24] found that higher TLC levels are associated with worse outcomes in sepsis patients, supporting the finding that TLC is higher in deceased patients. Monneret and Venet [26] also noted that persistent leukocytosis is a marker of poor prognosis in sepsis.

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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CONCLUSION

The expression of HLA-DR on monocytes decreases during the immunosuppression state of sepsis that increases the risk of secondary infection and poor prognosis.

The percentage of mHLA-DR expression on monocytes is lower in patients diagnosed with sepsis than in healthy subjects.

The level of Human Leukocyte Antigen-DR expressed on monocytes (mHLA-DR) is an important biomarker in sepsis and can be used as a predictive tool for the clinical outcome of patients.

REFERENCES

1. World health organization (WHO). (2020). Sepsis
2. Centers for disease control and prevention, (CDC). (2021), What is Sepsis.
3. Huang M, Cai S, Su J. The Pathogenesis of Sepsis and Potential Therapeutic Targets. Int J Mol Sci. 2019;20(21):5376. Published 2019 Oct 29. doi:10.3390/ijms20215376. MD Degree Thesis/MFA/ISRO/Faculty of Medicine/ASU/2022 8
4. Greco M, Mazzei A, Suppressa S, et al. Human Leukocyte Antigen-DR Isotype Expression in Monocytes and T Cells Interferon-Gamma Release Assay in Septic Patients and Correlation with Clinical Outcome. J Clin Med Res. 2021;13(5):293-303. doi:10.14740/jocmr4474.
5. D'Elia RV, Harrison K, Oyston PC, et al. Targeting the "cytokine storm" for therapeutic benefit. Clin Vaccine Immunol. 2013;20(3):319-327. doi:10.1128/CVI.00636-12
6. Srisawat N, Tungsanga S, Lumlertgul N, et al. The effect of polymyxin B hemoperfusion on modulation of human leukocyte antigen DR in severe sepsis patients. Crit Care. 2018;22(1):279. Published 2018 Oct 26. doi:10.1186/s13054-018-2077-y.
7. Holoshitz J. The quest for better understanding of HLA-disease association: scenes from a road less travelled by. *Discov Med*. 2013;16(87):93-101
8. Choo SY. The HLA system: genetics, immunology, clinical testing, and clinical implications. *Yonsei Med J*. 2007;48(1):11-23. doi:10.3349/ymj.2007.48.1.11
9. Van der Poll T, van de Veerdonk FL, Scicluna BP, Netea MG. The immunopathology of sepsis and potential therapeutic targets. *Nat Rev Immunol*. 2017;17(7):407-420. doi:10.1038/nri.2017.36
10. Liu Y, Wang R, Cheng J, et al. Ratio of serum procalcitonin to monocytic HLA-DR as a reliable

parameter in prognosis prediction of sepsis. Clin Chim Acta. 2021; 519:94-100. doi: 10.1016/j.cca.2021.04.011.

11. Quirant-Sánchez B, Plans-Galván O, Lucas E, Argudo E, Martinez-Cáceres EM, Arméstar F. HLA-DR Expression on Monocytes and Sepsis Index Are Useful in Predicting Sepsis. *Biomedicines*. 2023;11(7):1836. Published 2023 Jun 26. doi:10.3390/biomedicines11071836
12. Nedeva C, Menassa J, Puthalakath H. Sepsis: Inflammation Is a Necessary Evil. Front Cell Dev Biol. 2019; 7:108. Published 2019 Jun 20. doi:10.3389/fcell.2019.00108.
13. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA*. 2016;315(8):801–810. doi:10.1001/jama.2016.0287
14. Tille PM (2017): Traditional cultivation and Identification. In Bailey and Scott's Diagnostic Microbiology. Fourteenth edition. St. Louis, Missouri: Elsevier. 86-112.
15. Rahman M (2006). Introduction to flow cytometry. AbD SEROTECH
16. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol*. 2013;13(12):862-874. doi:10.1038/nri3552
17. Iskander KN, Osuchowski MF, Stearns-Kurosawa DJ, et al. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. *Physiol Rev*. 2013;93(3):1247-1288. doi:10.1152/physrev.00037.2012
18. Min SY, Yong HJ, Kim D. Sex or gender differences in treatment outcomes of sepsis and septic shock. *Acute Crit Care*. 2024;39(2):207-213. doi:10.4266/acc.2024.00591
19. Anush MM, Ashok VK, Sarma RI, Pillai SK. Role of C-reactive Protein as an Indicator for Determining the Outcome of Sepsis. *Indian J Crit Care Med*. 2019;23(1):11-14. doi:10.5005/jp-journals-10071-23105
20. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [published correction appears in JAMA. 2016 May 24-31;315(20):2237]. *JAMA*. 2016;315(8):762-774. doi:10.1001/jama.2016.0288
21. Gonçalves-Pereira J, Povoa PR, Lobo C, Carneiro AH. Bloodstream infections as a marker of community-acquired sepsis severity. Results from the Portuguese community-acquired sepsis study (SACiUCI study). *Clin Microbiol Infect*. 2013;19(3):242-248. doi:10.1111/j.1469-0691.2012.03776.x
22. Afzal MS, Nandan Chennuri R, Naveed H, et al. Comparison of Clinical Outcomes Between Culture-Positive and Culture-Negative Sepsis and Septic Shock Patients: A Meta-Analysis. *Cureus*. 2023;15(2):e35416. Published 2023 Feb 24. doi:10.7759/cureus.35416
23. Landelle C, Lepape A, Voirin N, et al. Low monocyte human leukocyte antigen-DR is independently associated with nosocomial infections after septic shock. *Intensive Care Med*. 2010;36(11):1859-1866. doi:10.1007/s00134-010-1962-x
24. Jang JY, Yoo G, Lee T, Uh Y, Kim J. Identification of the robust predictor for sepsis based on clustering analysis. *Sci Rep*. 2022;12(1):2336. Published 2022 Feb 11. doi:10.1038/s41598-022-06310-8
25. Leijte GP, Rimmelé T, Kox M, et al. Monocytic HLA-DR expression kinetics in septic shock patients with different pathogens, sites of infection and adverse outcomes. *Crit Care*. 2020;24(1):110. Published 2020 Mar 20. doi:10.1186/s13054-020-2830-x
26. Monneret G, Venet F. Sepsis-induced immune alterations monitoring by flow cytometry as a promising tool for individualized therapy. Cytometry B Clin Cytom. 2016;90(4):376- 386. doi:10.1002/cyto.b.21270.
27. Perry SE, Mostafa SM, Wenstone R, Shenkin A, McLaughlin PJ. Is low monocyte HLA-DR expression helpful to predict outcome in severe sepsis?. *Intensive Care Med*. 2003;29(8):1245-1252. doi:10.1007/s00134-003-1686-2