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Flow Cytometry of CD64, HLA-DR, CD25, and TLRs for Diagnosis and Prognosis of Sepsis in Critically Ill Patients Admitted to the Intensive Care Unit: A Review Article

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Abstract

Sepsis is an important health problem with a high burden on health systems. Finding new aspects of immune system function in sepsis showed a new role for flow cytometry in the diagnosis of sepsis. We made a review on the role of CD64, HLA-DR, CD25, and TLRs as more useful flow cytometric tools in diagnosing sepsis, both in adults, and neonates. According to our results, we concluded that for diagnosis and treatment of the septic, flow cytometry can play an important role so that it can be used as a novel method in individualized treatment of septic patients based on their immune system situation.

Keywords: Flow Cytometry, Sepsis, CD 64, HLA-DR, CD 25, Toll Like Receptor

1. Context

As the leading cause of mortality in critically ill patients, sepsis is a complex syndrome (1). It can cause severe organ failure and complicate an infection to a devastating, poor prognosis septic shock (2). By aging of population worldwide, the incidence of sepsis is increasing and it is a major healthcare problem today. Despite progress in medical cares, mortality rate from sepsis and septic shock is still as high as 30% to 50% (3). Early diagnosis of sepsis ensures timely treatment, which can reduce the organ failure and mortality. However, there are many challenges in sepsis and septic shock diagnosis. Blood culture, as a gold standard diagnostic way, needs time to confirm the diagnosis and many other biochemical markers, which are used as a screening tool, such as pro calcitonin (PCT), are not sufficiently specific (4, 5). Thus, there is a need of a diagnostic tool for timely diagnosis of sepsis.

Understanding the pathophysiology of sepsis can help to find a way. As a classic definition, sepsis is a systemic inflammatory response to an infection (6). Its pathogenesis is based on complex networks of pro-inflammatory and anti-inflammatory processes (7). For years, tissue damage that is caused by exaggerated pro-inflammatory activation has been thought to be the core pathophysiologic proses in sepsis. As the treatments directed to reducing this proses failed to improve the outcome of sepsis, this hypothesis has been questioned. Recently, an immunosuppressed hypo inflammatory state, which starts at very early hours of sepsis is directed as an important factor in the sepsis outcome (8). This hypo inflammatory state, which recently termed as persistent inflammation/immunosuppression and catabolism syndrome (PICS), can extend to late phases of sepsis and can continue even after patient discharge from the hospital (9, 10). We know that both pathogen associated molecular patterns (PAMPs) and damage associated molecular patterns (DAMPs) start a complex intracellular signaling system that can cause inflammatory responses to an infection (11). Despite the fact that the exact cause of PICS is not clear, DAMPs are more likely to have the major role (12). Based on these findings, Singer et al. changed the classic definition of sepsis to a new one. They defined sepsis as "life threatening organ dysfunction caused by a dysregulated host response to infection" (13). Changes in circulatory cytokines and surface markers can help understanding the mechanism of systemic response to infection and

finding new diagnostic tools.

Since late 1960s, Flow cytometry has been a confidential diagnostic tool in the diagnosis of immune related disorders for decades. Flow cytometry is a technic for profiling and sorting of cells or other particles by illuminating them when they flow in front of a light source. The technic is based on light scattering features of the cells and particles when a LASER beam illuminate them (14). Nowadays, flow cytometry is more accessible for physicians and new technics help reduce the time needed for results. Furthermore, use of portable and bed-side flow cytometry will facilitate the use of this tool in critical ill patients (15). By these improvements it can play a new role in early diagnosis of sepsis.

In this study we review the use of cluster of differentiation 64 (CD64), human leukocyte aAntigen – antigen D related (HLA-DR), and CD25 as the three more frequently used markers in flow cytometry for critical care.

2. Evidence Acquisition

To get the most relevant data about flow cytometry in sepsis work out, we used reliable evidence by searching MEDLINE, PubMed, Scopus, DOAJ, and Cochrane databases. Searching for systematic reviews, original articles, clinical trials, and reviews done by authors using flow cytometry, sepsis, CD25, CD64, HLA-DR, toll like receptor, monocytes, neutrophils, lymphocytes, dendritic cells, and interleukins as keywords.

Studies in other languages rather than English and studies older than 15 years were excluded from this study due to language the limitation of authors and in trying to achieve most recent data. A total of 347 articles have been found based on title. Duplicated articles were excluded and 339 remaining studies were criticized by two individual authors and their quality assessed. Not relevant studies and low-quality studies were excluded from the study. In case of disagreement regarding a studies quality, a third author criticized the study and the choice of inclusion or exclusion made by him. A total of 35 studies enrolled in this review finally (Table 1).

Our study, as a simple (narrative) review, has some limitations. When we could not access full text or abstract of a study, we exclude it and we also had a limitation of language. The strong point of our study was using most recent articles and also studying a new subject to solve an old problem.

3. Results

3.1. CD64

Neutrophils has an important role in host defense against infections and are essential components in immunity response (29). CD64, a high affinity Fcγ receptor, found on monocyte/macrophage surface on normal situations and only less than 2000 CD64 molecules found on normal neutrophils. In systemic inflammatory response syndrome (SIRS), this molecule upregulates on the surface of neutrophils (15, 30, 31). The expression of CD64 on neutrophils surface starts on a very early phase of immune response to bacterial infection and increase in one hour (32). This expression will dramatically decrease within 48 hours after removal of stimulation and the level of CD64 returns to normal in seven days (17). Its stable expression for more than 24 hours in room temperature and simplicity of its detecting by flow cytometry, makes an interest to studying value of this molecule in diagnosis of sepsis (15). In a meta-analysis, Cid et al. concluded that CD64 can be a useful marker in early diagnosis of bacterial infection (16). Wang et al. found CD64 75% sensitive and 86% specific in a meta-analysis study in 2015. They concluded that although CD64 is not perfect in diagnosis of sepsis, it can have a positive role in this purpose (17). In combination with other markers such as procalcitonin, the accuracy of CD64 can be improved (5). Recently, Bauer et al. studied 219 adult patients in a case control study between 2012 to 2014. They concluded that a combination of CRP, PCT, and CD64 can improve the accuracy of diagnosis in septic patients when infection has been yet confirmed (18). In a single center prospective study in 2012, Bae et al. found a prognostic value for CD64 in septic critically ill patients. They studied 74 ICU patients with severe sepsis or septic shock from different infection sources. Higher expression of CD64 in first day of admission correlates with a better outcome (19). Previously, Danikas et al. showed a same correlation between CD64 expression and prognosis of sepsis (33). Coberly et al. studied 100 patients with suspected sepsis and found an excellent negative predictive value for CD64. They found 100% sensitivity and 100% negative predictive value, although specificity was low in this study (28% specificity) (20). Muzlovic et al. also found CD64 a predictor in VAP induced sepsis and a 30-day prognosis indicator in patients. They found a lower index of CD64 as an indicator of better prognosis and lower mortality rate. Although the study was a pilot study with only 32 participants (21).

3.2. HLA-DR

HLA-DR predominantly express on the surface of monocyte/macrophages, dendritic cells, and B cells and play a crucial role in adoptive immune response. It's a MHC class II molecule and its surface expression in essential for antigen presenting function of these cells (34). Its significant role first described in patients undergoing organ transplantation by Reinke and Volk (34, 35).

Table 1. Highlights of Studies in Flow Cytometry

Bio Marker/Authors	Highlights of Study
CD64	
Cid et al. (16)	Found CD64 a useful marker in diagnosing sepsis.
Wang et al. (17)	Found CD64 75% sensitive and 86% specific.
Bauer et al. (18)	Combination of CRP, PCT and CD64 can improve the accuracy of diagnosis in septic patients.
Bae et al. (19)	Higher expression of CD64 in first day of admission correlates with a better outcome.
Coberly et al. (20)	They found an excellent negative predictive value for CD64.
Muzlovic et al. (21)	They found lower index of CD64 as an indicator of better prognosis and lower mortality rate.
HLA-DR	
Winkler et al. (22)	They found a significantly lower expression of HLA-DR in peripheral blood of septic patients.
Lekkou et al. (23)	Lower HLA-DR expression in non-survivors versus survivors of sepsis.
Bauer et al. (18)	They did not find HLA-DR expression frequency a good discriminator for sepsis.
Zouiouich et al. (24)	They used a new tactic of flow cytometry to reduce the time of measurement.
CD25	
Llewelyn et al. (25)	They found 83% sensitivity and 83% specificity in distinguishing sepsis from non-infective SIRS.
Matera et al. (26)	They found 87.5% sensitivity and 75% specificity in first day of admission and 87.5% sensitivity and 77.8% specificity in seventh day of admission for sCD25.
García de Guadiana-Romualdo et al. (27)	They found a similar performance for sCD25 than procalcitonin.
Monneret et al. (28)	They found a higher expression of CD25 in sepsis non-survivors.

More than 30 years ago, Polk et al. reported an association between the low expression of HLA-DR and development of sepsis (36). Since that time, many authors discussed the role of HLA-DR in diagnosis and prognosis of sepsis (36, 37). Nowadays Low HLA-DR expression is considered as a reliable marker and a predictor of septic complications in critically ill patients (15, 36, 38). Low HLA-DR expression is also a prognostic indicator in this group of patients. Cheron et al. studied 105 trauma patients over a 23 months' period of time and showed that traumatic patients, with low expression of HLA-DR, had more infective complications and poor prognosis in comparison to patients with high or normal expression (37). Winkler et al. found a higher number of monocytes, however,with a significantly lower expression of HLA-DR in peripheral blood of septic patients (22). Lekkou et al. studied 30 severe sepsis patients in the ICU and found lower HLA-DR expression in non-survivors versus survivors of sepsis (23).

Although generally accepted as a reliable marker, there are still some controversies regarding its use in clinic. For example, in a prospective double-blind study of diagnostic accuracy for sepsis in ICU patients, Bauer et al. did not find HLA-DR expression frequency a good discriminator for sepsis (18). In addition, there are a couple of drawbacks in HLA-DR measurements. Cell staining must be done within two to four hours from sampling, which can restrict routine use of HLA-DR in daily practice (39). To solve this problem, use of table top fully automated cytometers can help clinicians. Zouiouich et al. used a Accellix cytometer for this purpose and showed that it has reliable and valid results in comparison with standard flow cytometers (24). This can make flow cytometry of HLA-DR an accessible diagnostic modality for intensivists and emergency medicine clinicians. Use of quantitative real-time polymerase chain reaction (qPCR) for detecting of HLA-DR can play a new role in sepsis work out and can be an alternative for flow cytometry (22).

3.3. CD25

CD25, an IL2 receptor alpha chain, is a surface marker of regulatory T cells and also expressed on activated effector T cells (25). Septic patients show a higher level of soluble CD25 (sCD25) than non-infective SIRS positive patients (40). Recently, soluble form of CD25 attract researchers as a new marker in sepsis. Llewelyn et al. showed the good performance of sCD25 as a sepsis marker in an early phase of admission in a study with 219 patients enrolled. They found 83% sensitivity and 83% specificity in distinguishing sepsis from non-infective SIRS (25). Matera et al. also found 87.5% sensitivity and 75% specificity in the first day of admission and 87.5% sensitivity and 77.8% specificity in seventh day of admission for sCD25. Soluble CD25 also showed a good sensitivity and specificity in predicting mortality (26). García de Guadiana-Romualdo et al. used CD25 as an

infection and sepsis marker in 152 patients with suspected sepsis and found a similar performance than procalcitonin (27). Monneret et al. found a higher expression of CD25 in sepsis non-survivors in comparison with survivors. They also describe that not septic patients had an even lower CD25 expression on their lymphocytes. The study also discussed HLA-DR low expression in septic patients (28).

3.4. Toll Like Receptors

Toll like receptors (TLR) have a fundamental role in host defense and also in pro inflammatory response to infection. These molecules belong to a family of surface receptors called pattern recognition receptors (PRR). Thirteen deferent TLRs have been found on the cell membrane or endolysosomal membranes of natural killer (NK) cells, monocyte, macrophages, and other cells of innate immunity (41). These receptors have a crucial role in recognition of invading microorganisms and introducing them to the immune system. In a pilot study, Holst et al. finds TLRs more sensitive and more specific than C-reactive protein for sepsis in ICU patients (41). Although studies showed difference of TLR expression on NK cells in septic and non-septic patients, however, there is not a conclusion on diagnostic or prognostic value of TLR studding by flow cytometry and more studies are needed (42, 43).

3.5. Flow Cytometry in Neonatal Sepsis

Neonatal sepsis has an incidence of 3 - 40 in 1000 live birth and a mortality rate ranges from 9% to 20%. Due to nonspecific clinical manifestations and lack of diagnostic criteria for neonatal sepsis, its early diagnosis is challenging (44). Blood culture as a standard diagnostic tool has some limitations; it is time consuming and also has a high rate of false positive and false negative results (45).

CD64 as a new marker to diagnosing neonatal sepsis has been studied by many researchers and different findings published in literature. Two important meta-analysis recently studied CD64 in neonatal sepsis. Dai et al. enrolled seven studies with 2237 neonates in their meta-analysis. They found a pooled sensitivity of 80% and a specificity of 83% and concluded that CD64 is a reliable marker for diagnosing neonatal sepsis (32). In contrast with this study, Shi et al. did not find CD64 a sensitive and specific marker for this purpose. Their meta-analysis enrolled 17 studies with 3478 participants and they found a pooled sensitivity and specificity of 77% and 74%, respectively (46).

HLA-DR also has been studied in neonatal sepsis. Kanakoudi-Tsakalidou et al. found a lower HLA-DR in neonates in comparison with adults. However, like adult septic patients, septic neonates had a lower expression of HLA-DR on their monocytes in comparison with healthy neonates. They studied 131 neonates and found low expression in both sepsis and RDS patients. No significant different has been found in term and preterm subgroups (47). Ng et al. also studied HLA-DR in neonatal sepsis, however, they did not find a difference in septic and healthy patients (48). In contrast Genel et al. found a significant lower HLA-DR in septic neonates and found a prognostic value for HLA-DR in this group of patients (49).

4. Conclusions

Flow cytometry can play an important role in sepsis work up and treatment and can be used as a novel method in individualized treatment of septic patients based on their immune system situation. It can be used as a prognostic tool, too. If we can prove that diagnosis of sepsis can improve by flow cytometry and it can reduce morality of patients with septic shock and sepsis, we can manage our patients more effectively considering almost low cost and short processing time for flow cytometry.

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Footnotes

Authors' Contribution: Study concept, design and supervision: Ata Mahmoodpoor and Hassan Soleimanpour; drafting of the manuscript: Seyedpouya Paknezhad, Sarvin Sanaie, Kamran Shadvar, Ali Akbar Ghamari, Ali Akbar Movassaghpour and Hadi Hamishehkar.

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