Burns have grown to be a significant public health issue in the last decade, with little more than a quarter of million deaths each year. Major burns trigger a significant inflammatory response that frequently progresses to a systemic response inflammatory syndrome, sepsis, and ultimately multiple organ failures. The inflammatory process defined by the recruitment of myeloid, T-cells, and the participation of several cytokines, chemokine, complement fractions, and growth factors is the primary mechanism involved in wound healing following burns. Inflammation is protein-based cytosolic complex that plays a part in modifying and enhancing the defense capability of the innate immune system when activated by metabolic stress or infection. This study aimed to investigate the correlation between bacterial infection in burn patients and the cluster level of differentiation (CD14) in their serum.
Introduction

Any damage occur in the skin or other organic tissue by heat is known as burn, but it can also be the result of radiation, electricity, friction, or contact with chemicals. Burns injuries have a high morbidity rate. Burns that do not result in death might result in the extended hospital stays, deformity, and handicap; this typically results in shame due to the scales, the rigid areas in the body, and rejection from the community [1]. Burn wounds offer the perfect habitat for the development of both naturally occurring and introduced opportunistic organisms. The leading factor in mortality and morbidity in burn patients is infection; it is a difficult problem for the burn experts in the hospital to solve. The altered immunity increases the infection risk which can results in sepsis. Following the initial therapy, 50-70% of burn patients die from the problems connected to infections [2]. Many studies have shown that subsets of innate and adaptive immune cells in sepsis (is a condition that can be fatal and is brought on by the body's immunological reaction to an infection. It results in multiple organ failures such as natural killer cells, dendritic cells, macrophages, T-lymphocytes, regulatory T-lymphocytes, and neutrophils that have the profound effects on immune-reactivity during acute insults or sepsis) is a condition that can be fatal and is brought on by the body's immunological reaction to an infection. It results in multiple organ failures such as natural killer cells, dendritic cells, macrophages, T-lymphocytes, regulatory T-lymphocytes, and neutrophils that have the profound effects on immune-reactivity during acute insults or sepsis [3], the precision of the prognosis of septic patients, the likelihood of subsequent infection and the management of septic squeal, which is of major importance [4]. Cluster of differentiation 14 (CD14) is a human protein made mostly by macrophage as a part of innate immune system [5]. CD14 helps to detect bacteria in the host body by binding lipopolysaccharide (LPS) in gram-negative bacteria where lipopolysaccharide binding protein (LPS) is present [6]. Similarly, binding the peptidoglycan (PG) in gram-positive bacteria [7] are causes of infection in burns. The most prevalent microorganisms that cause infection in burn patients and can result in death could be bacteria, fungi, and viruses [8].

1. Gram positive bacteria: *Staphylococcus aureus, Streplococcus* spp., and *Enterococcus* spp.
2. Gram negative bacteria: *Pseudomonas aeruginosa, acinetobacter baumannii, klebsiellapneumoniae*, and *enterobacteriaceae* species.

Presepsin (CD14): On the surface of immune cells including monocytes and macrophages, CD14 functions as a receptor for lipopolysaccharide (LPS). Following the interaction with infectious pathogens, N-terminus of CD14 is cleaved and released into circulation as soluble CD14 subtype, one of which has been identified as the 13kDa glycol peptide presepsin (PSEP). Although its physiological function is still unknown, it is thought to be related to bacterial phagocytosis, microorganisms, and lysosomal cleavage. Presepsin has been suggested as a potential biomarker for sepsis diagnosis because serum levels rise earlier in the immunological response to sepsis than procalcitonin or IL-6. In multiple researches by using meta-analysis [9], presepsin has been demonstrated to be a reliable diagnostic marker for sepsis. An increase in presepsin levels is associated with the bacteremia status of SIRS (Systemic Inflammatory Response Syndrome) patients who are referred to the emergency room, according to a prospective study [10]. The cell wall (LPS) in gram-negative bacteria and the (PG) in gram-positive bacteria both include the receptor peresepsin. Thus, presepsin is recognized as a helpful biomarker for the sepsis diagnosis and differentiating bacterial infection, but further study is required to determine its utility for prognostication [5].

Materials and methods

Sample collection

This study was conducted from the 1st of November 2021 to the end of February 2022. The wound samples and blood samples were collected from sixty Iraqi patients who admitted to the hospitals after burns injury (based on moral considerations that patients have approved). The number of patients included thirty males and thirty females, and thirty healthy participants as controls. The samples of blood and wounds were collected from specialized burns Hospital, Medical City, Al-
Yarmouk Teaching Hospital and Al Karama Teaching Hospital. The patients were suffering from burns in different degrees; the most frequent degree was the third degree.

The immunological study
The blood specimens were obtained per single day from the admission day to the discharge day, or the death day, with follow up to the patient situation at 24 hours. 5ml of blood was collected. Thirty patients were died after admission to the hospitals with different periods of stay. Thirty patients were discharge from hospitals after healing and they were returned to the healthy situation. Blood was taken within the final 24 hours before deaths. Blood samples from healthy individuals were acquired for the final 30 samples as a baseline. For 10 minutes, all of the samples were centrifuged at 3000 rpm. At -18°C, the isolated serum was collected and frozen.

The bacterial study
The swab samples were 180 swabs taken from different areas of burn after one day of admission. 90 isolate swabs were with the bacteria growth (pathogenic bacteria). 90 isolate swabs were with no growth. All the swabs were cultured on Chocolate agar, Blood agar, MacConky agar, and Sabouraud agar.

Identification of bacterial isolates by using API 20 (Analytical Profile Index 20) system
Representative colonies from MacConky agar plates were sub-cultured on API20E micro tube system to identify the isolates. 20 typical biochemical tests (ONPG, ADH, LDC, ODC, CIT, H₂S, URE, TDA, IND, VP, GEL, GLU, MAN, INO, SOR, RHA, SAC, MEL, AMY, and ARA) can be run with this technique from single colony grown on the plate medium. In this technique, each test was conducted in side a sterilized plastic micro tube that contained the necessary substrates and was attached to an impermeable plastic strip (gallery). There were 20 micro tubes in each gallery.

Identification of bacteria by VITEK-2 Compact system
Through biochemical interactions between the suspended bacterial isolates in their solutions and the medium on the identification card, the VITEK-2 system detects the isolates. The bacterial isolates were plated onto MacConky agar and cultured at 37 °C for an overnight period. Then, a single colony was removed and placed in solution. By using VITEK Densi-Chek (BioMrrieux), the turbidity of the bacterial suspension tubes and VITEK2 ID card were manually put into the VITEK2 machine. The steps of the software were then carried out according to the directions provided by the manufacture (BioMérieux, France) [11].

Human soluble cluster of differentiation 14 (sCD14)
Enzyme-Linked Immuno sorbent assay kit
Assay principle
The quantitative sandwich enzyme immunoassay is used in this assay which is its basic working principle. A micro plate had previously been covered with CD14-specific antibody. The immobilized antibody binds to any CD14 presented as standards and samples are pipetted into the wells. A biotin-conjugated antibody specific for CD14 is added to the wells after any unbound materials got rid by washing in water. Avidin conjugated horseradish peroxidase (HRP) is added to the wells after washing (because the conjugate is what attaches the Ag-Ab molecule to the anti-Ab, doing a color solution after adding TMB requires the conjugate). Then, a substrate solution is added to the wells after a wash to remove any unbound avidin-enzyme reagents and color develops in proportion to the amount of CD14 bound in the initial phase. Next, the intensity of the color is measure by a spectrophotometer, while its development is halted.

Results
Comparison of the bacterial isolation of the study groups
Apart from burn-free controls group whose without wound swabs and no one has a positive growth of template, gram negative culture of wound swab was more than gram positive cultures among cases groups of both dead-cases and healed-cases groups (98% vs. 2%) and (85% vs. 7.5%), respectively. However, only 7.5% of healed-cases group were identified to have no positive growth of template (Figure 1).
Moreover, the isolated bacteria among dead-cases group were mainly *Acinetobacter baumannii* (5; 16.7%), followed by *Pseudomonas aeruginosa* (2; 6.7%). However, other isolations were in combination of bacteria as of *Acinetobacter baumannii* with *Klebsiella pneumoniae* (12; 40%), followed by *Pseudomonas aeruginosa* with *Klebsiella pneumoniae* (5; 16.7%), *Pseudomonas aeruginosa* with *Acinetobacter baumannii* (2; 6.7%), *Pseudomonas aeruginosa*, *Acinetobacter baumannii* with *Klebsiella pneumoniae* (3; 10%), and *Staphylococcus aureus* with *Acinetobacter baumannii* (1; 3.3%), respectively. On the other hand, a single bacterial isolation was predominant among healed-cases group rather than bacterial combinations, mainly *Klebsiella pneumoniae* (8; 26.7%) followed by *Acinetobacter baumannii* (6; 20%), *Staphylococcus aureus* (3; 10%), *Pseudomonas aeruginosa* (2; 6.7%), and *Proteus mirabilis* (1; 3.3%).

The main combination among healed-cases was by *Proteus mirabilis* with *Klebsiella pneumoniae* (3; 10%) followed by *Acinetobacter baumannii* with *Klebsiella pneumoniae*, and that of *Pseudomonas aeruginosa* with *Acinetobacter baumannii* (each 2; 6.7%), respectively (Figure 2).
Comparison of immunological parameters among the study groups

The mean levels of cluster of differentiation 14 (CD-14) was found to be significantly higher among dead-cases group than that of healed-cases groups which subsequently was higher than that of controls (7.140 ± 3.3903 vs. 3.200 ± 0.7991 vs. 1.863 ± 0.6984), respectively (F= 53.666, df: 2, 87, P= 0.000) with significant differences of 5.277 and 1.337 from control group, respectively (Figure 3).

Table 1: Comparison of CD14 levels between research groups (n=90)

<table>
<thead>
<tr>
<th>Study group</th>
<th>Immunological parameter of cluster of differentiation 14 (CD-14)</th>
<th>Mean ± SD</th>
<th>Mean difference&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Significance&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td></td>
<td>1.863 ± 0.6984</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healed-cases</td>
<td></td>
<td>3.200 ± 0.7991</td>
<td>1.337</td>
<td>F = 53.666</td>
</tr>
<tr>
<td>Dead-cases</td>
<td></td>
<td>7.140 ± 3.3903</td>
<td>5.227</td>
<td>df: 2.87</td>
</tr>
</tbody>
</table>

<sup>a</sup>Mean difference from mean value of controls group

<sup>b</sup>One-way ANOVA test

![Figure 3: CD14 Comparison among the study groups (n=90)](image)

The 14<sup>th</sup> cluster of differentiation (CD14) as a predictive diagnostic marker for lethality risk among burned patients

Similarly, the optimal cutoff value of the 14<sup>th</sup> cluster of differentiation (CD14) for detecting burned patients with a high death risk was 3.950 with sensitivity of 90%, and specificity of 83.3% and was correctly predicted by the regression model of 85% with excellent area beneath the ROC (AUC) curve of 0.943 ± 0.028 (P= 0.000).

Table 2: CD-14 value as a risk indicator for mortality in a samples of burned patients (n=60)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (Sn)</th>
<th>Specificity (Sp)</th>
<th>Accuracy</th>
<th>Area Under the curve (AUC)</th>
<th>Significance (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster of differentiation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(CD-14)</td>
<td>90</td>
<td>83.3</td>
<td>85</td>
<td>0.943</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Discussion
In the current research, the results revealed that out of 90 samples of wound swabs were gram-negative bacteria more than the gram-positive bacteria. The dead-cases 98% (GN) compared with 2% (GP), while the cured-cases were 85% (GN) compared with 7.5% (GP) and 7.5% was with no growth.

These results were similar to study performed in Iraq by [12] which revealed that the measured 130 samples analyzed 72.3% of microorganisms were growing in the culture, and 27.7% of culture was with no growth. The gram-negative bacteria were 99% of cultures. Another study in Ghana by [11] showed the results of 50 samples cultured, 43(86%) were with growth, and 7(14%) were with no growth. The gram-negative bacteria were 97.7% and the gram-positive bacteria were 2.3%. These results may be due to the bacteria resistance to the antimicrobial agents and the hospital acquired infection after the admission.

On the other hand, the study reported by [14] in Iraq was inconsistent with the present study by results of 500 samples cultured the gram-positive bacteria were 277 (55.4%) and the gram-negative bacteria were 223 (44.6%).

These bacteria originate from the patients surface flora. The argument for doing the operation as soon as feasible is supported by the fact that delaying burn wound infection excision increases bacterial load, particularly gram-positive bacteria. In our study, the most frequent isolated bacteria in the dead-cases was Acinetobacterbaumannii which presence (16.7%) in the single isolated bacteria, followed by Pseudomonas aeruginosa which present (6.7%) of the single isolated bacteria. These results were corresponded to a study by [15] in Iraq that revealed 90 samples were taken from patients in 2 hospitals in Baghdad city and the results was 65 samples (72.2%) were Acinetobacterbaumannii and the remain samples were the other microorganisms, and a study by [16] in Turkey which was a 3-years record at Van Training and Research Hospital in Van. The total number of bacterial isolates was 250 from 179 patients, the Acinetobacterbaumannii was (23.6%), after it coagulase negative Staphylococci (13.6%), and then Pseudomonas aeruginosa (12%), Staphylococcus aureus (11.2%) and Escherichia coli (10%). Whereas the other studies demonstrated that the Pseudomonas aeruginosa was the most prevalent bacteria in burn infections, as observed in [17].

These results reveled that each burn facility should find the precise pattern of microbial colonization of burn wounds, changes in the dominant flora with time, and antimicrobial sensitivity profiles. Without waiting for culture results, this would make it possible to address impending sepsis crises with appropriate
systemic antibiotics used in practice, significantly reducing infection-related morbidity and mortality.

CD14 (cluster of differentiation 14) is a glycoprotein that serves as a receptor for endotoxin complexes and activates signal transduction pathways thought to be involved in a systemic inflammation. Serum CD14 levels have a positive correlation with sepsis severity and have been recognized as the sepsis precursor [18]. In this study, the CD14 level recorded a high value in the group of dead-cases compared with healed-cases and the control cases recorded the lower value (Table 1).

A study in Iraq by [19] demonstrated that the CD14 value in patients with bacterial infection is significantly higher than healthy people. Furthermore, another study by [20] showed the same results a higher value of CD14 in patients with a septic shock. The same results are shown in [21] which revealed that the CD14 value was elevated by the bacterial infection.

**Conclusion**

With these findings, the authors concluded that the presepsin level, rather than the inflammation degree should reflect the infection severity. Patients who were septic had the higher SCD14 levels, which did not differ significantly between gram-positive and gram-negative infections. Clinical experimental results support the concept proof for the high specificity of this biomarker. Presepsin appears to be the most promising novel biomarker for sepsis in the early diagnosis and a superior predictive biomarker.

**APPREVIATES**

ADH: Decarboxylation of amino acid arginine by arginine dihydrolase
AMY: Fermentation of amygdalin (glycoside)
ARA: Fermentation of arabinose (pentose sugar)
CIT: Utilization of citrate as the only carbon source
GEL: Test for the production of the enzyme gelatinase which liquefies gelatin
GLU: Fermentation of glucose (hexose sugar)
H₂S: Production of hydrogen sulfide
IND: Indole Test-production of indole from tryptophan by the enzyme tryptophanase
Reagent- Indole is detected by the addition of Kovac's reagent.
INO: Fermentation of inositol (cyclic polyalcohol)
LDC: Decarboxylation of amino acid lysine by lysine decarboxylase
MAN: Fermentation of mannose (hexose sugar)
MEL: Fermentation of melibiose (disaccharide)
ODC: Decarboxylation of amino acid ornithine by ornithine decarboxylase
ONPG: Test for β-galactosidase enzyme by hydrolysis of the substrate o-nitrophenyl-b-D-galactopyranoside
RHA: Fermentation of rhamnose (methyl pentose sugar)
SAC: Fermentation of sucrose (disaccharide)
SOR: Fermentation of sorbitol (alcohol sugar)
TDA (Tryptophan deaminase): Detection of the enzyme tryptophan deaminase: Reagent- Ferric Chloride.
URE: Test for the enzyme urease
VP: The Voges-Proskauer test for the acetoin detection (acetyl methylcarbinol) produced by the glucose fermentation by bacteria utilizing the butylene glycol pathway.

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**Authors' contributions**

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

**Conflict of Interest**

There are no conflicts of interest in this study.

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