AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Novel septicemia biomarker :HDL (High Density Lipoproteins)

Al-Zaidawi, Sejad [1]

1:Al-Sadr teaching hospital, burn unit, Maysan, Iraq

ABSTRACT

Sepsis is a leading cause of mortality in critically ill patients especially in burn unit. Delay in diagnosis and initiation of antibiotics treatment have been shown to increase mortality. This study aimed to find out a novel septicemia biomarker, more practical and easy. 48 patients had been admitted into burn unit during 4 months, 22 patients of them (46%) were complained of septicemia. HDL (high density lipoproteins) had been measured for patients in admitted to burn unit every other day to measure the correlation of HDL concentration and septicemia. HDL was a very good indicator for septicemia due to the wide range of normal value (40-60 mg/dl).Number of patients was 48 patients, 22 of them developed septicemia about 46%. 11 patients of the 22 patients are died (50%). Also it is clear to notice that all patients died were with (HDL = <5); 1 or 2 days before dying, all patients with HDL < 15 were with a positive blood culture, and all patients with septicemia when their HDL value increased 5 scales are cured of the infection, i.e. from < 5 to 10 for example. It is very important to find that patients with HDL value < 15 with abnormal triglyceride value (more than 200) were with high risk of developing septicemia HDL value in burn unit is the best biomarker for septicemia and it seems to give a map for the severity of infection and accurate time of initiation and stopping of antibiotics treatment.

Key words: HDL, septicemia, burn, Maysan and Iraq.

*Corresponding Author Email: Sajadsa52@yahoo.com

Received 24 August 2013, Accepted 05 September 2013

Please cite this article in press as: Sejad AL. *et al.*, Novel septicemia biomarker: HDL (High Density Lipoproteins). American Journal of PharmTech Research 2013.

INTRODUCTION

Sepsis is a leading cause of death in critically ill patients despite the use of modern antibiotics and resuscitation therapies[1]. The septic response is an extremely complex chain of events involving inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities[2,3] .The diagnosis of sepsis and evaluation of its severity is complicated by the highly variable and non-specific nature of the signs and symptoms of sepsis[4]. However, the early diagnosis and stratification of the severity of sepsis is very important, increasing the possibility of starting timely and specific treatment[4,5]

Biomarkers can have an important place in this process because they can indicate the presence or absence or severity of sepsis [7,8] and can differentiate bacterial from viral and fungal infection, and systemic sepsis from local infection. Other potential uses of biomarkers include roles in prognostication, guiding antibiotic therapy, evaluating the response to therapy and recovery from sepsis, differentiating Gram-positive from Gram-negative microorganisms as the cause of sepsis, predicting sepsis complications and the development of organ dysfunction (heart, kidneys, liver or multiple organ dysfunction). However, the exact role of biomarkers in the management of septic patients remains undefined [9] C-reactive protein (CRP) has been used for many years [10,11] but its specificity has been challenged[12] Procalcitonin (PCT) has been proposed as a more specific [13] and better prognostic [14] marker than CRP, although its value has also been challenged [15] It remains difficult to differentiate sepsis from other non-infectious causes of systemic inflammatory response syndrome [16] and there is a continuous search for better biomarkers of sepsis.

HDL as novel sepsis biomarker

High-density lipoprotein(HDL) play an important role in immunity and during infections and sepsis. HDL contains free or esterified cholesterol, phospholipids, triglycerides, and various proteins, including apolipoproteins, enzymes, and transfer proteins. The most abundant HDL apolipoproteins are apoA-I and apoA-II; less abundant are apoC, apoE, apoD, and apoJ. HDL enzymes include lecithin:cholesterolacyltransferase (LCAT), serum paraoxonase-1 (PON1)[17,19] , and platelet-activating factor acetylhydrolase (PAF-AH)[20] Transfer proteins include cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP). Furthermore, chromatography and mass spectrometry have revealed many other proteins in HDL [21,22] HDL particles can be sub classified into small discoidal HDL (pre-β_1 HDL and pre-β_2 HDL), intermediate spherical HDL_3 (HDL_{3c}, HDL_{3b}, and HDL_{3a}), and large, cholesterol-rich spherical HDL_2 (HDL_{2a} and HDL_{2b}) [23-26] Large HDL particles interact with liver scavenger receptors class

B type 1 (SR-B1), which ensures the delivery of cholesterol to the liver [27] Intermediate HDL3 induces cholesterol efflux through the ATP-binding cassette transporter G1 (ABCG1) [28] Small HDL particles promote cholesterol efflux through the ATP-binding cassette transporter A1 (ABCA1) [29] Accumulating evidence suggests that in addition to reverse transport of cholesterol from the periphery to the liver, HDL plays a major role in vasodilation and in the reduction of LDL oxidation [30] inflammation, apoptosis, thrombosis, and infection[31] During infection, both innate and adaptive immunities are involved in the inflammatory process and the immune response. Innate immunity is a nonspecific defense mechanism comprising cellular and humoral responses. The cellular response includes antigen-presenting cells such as macrophage and dendritic cells. The humoral response includes various effectors, such as the complement cascade or soluble pattern recognition receptors (PRRs). Adaptive immunity is an antigen-specific defense mechanism against foreign antigens or pathogens. The principal effectors of adaptive immunity are B lymphocytes (humoral response) and T lymphocytes (cellular response).

Methods

Blood samples are taken subsequently from patients to measure the HDL value every other day, at the same time clinical signs and symptoms of infection and septicemia are observed from admission till discharge of all patients during period from April to august 2013.

These blood samples are subjected to centrifuge for 5 minutes to extract serum. These serumswere transferred into special tube of ARCHITECT® C 4000 system which is automatically added reagent A &B of HDL to measure HDL value. This operation last for 20 minute to get a report for the HDL value with the normal range which is (40- 60 mg/dl). At the same time value of triglyceride was measured for the same sample with the normal range which is (0- 149 mg/dl) .the two results were printed in one report.

Another blood samples were cultured by VITEK 2® system to predict the septicemia depending on clinical evidences. These samples are taken by special container of VITEK 2®system.

Systems used in the research:

1: VITEK 2®:

The wide variety of VITEK® 2 identification cards (ID) and antibiotic susceptibility testing (AST) cards provides testing flexibility. This unique feature helps the laboratory control expenses and eliminates unnecessary testing. The VITEK 2®System and Test Cards originated with the NASA space program to identify infections in astronauts. This breakthrough innovation led to today's VITEK 2® technology. The VITEK 2® card is the size and shape of a playing card and contains 64 microwells. Each well contains identification substrates or antimicrobials.

VITEK 2® offers a comprehensive menu for the identification and antibiotic susceptibility testing of organisms.

The VITEK 2 Test Card is sealed, which minimizes aerosols, splattering, spills, and personal contamination. Disposable waste is reduced by more than 80% over microtiter methods.

Antibiotic Susceptibility Testing (AST):

Gram positive antimicrobial susceptibility testing (AST) cards, Gram negative antimicrobial susceptibility testing (AST) cards, Yeast antimicrobial susceptibility testing (AST) card

2: ARCHITECT® system:

a new diagnostic instrument - clinical chemistry analyzer which performs diagnostic tests that monitor general health including a patient's levels of sodium, potassium, chloride and organ function. This system from the American Association For Clinical Chemistry's Clinical Lab Expo in Chicago, Abbott®.

RESULTS AND DISCUSSION

Due to the increase in the mortality rate caused by sepsis , also septicemia consider to be the leading cause of mortality in burn unit ,it is very important to find out a biomarker for septicemia This biomarker should be easy, cheap, and accurate. During our research it is very clear to find a relationship between HDL value and developing of septicemia and SIRS.

Forty eight patients were admitted into burn unit at Al-Sadr teaching hospital in province Maysan, Iraq from April till august 2013 with BSA more than 15%(from 15 % – 90%), their age was (25_+24 years), most of cases by direct flame (74%), 40% of them were self-harming which were with BSA% more than 60% unfortunately all of them developed SIRS and most of them are died.

HDL value was dramatically decreased in burned patient, correlated to BSA% and degree of burn. HDL value and triglyceride were measured for all patients each other day with intensive fellow up of clinical status of patients. When HDL value was (<15 mg/ dl), with high triglyceride (> 150 mg/dl), blood culture was positive. It was 22 patients developed septicemia (blood culture was positive) concomitant with HDL (>15 mg/dl) and triglyceride (> 150 mg/dl) see (table: 1), eleven of them developed SIRS and died, with HDL value (<5 mg/dl) and triglyceride value (>200 mg/dl).

It was continue to measure HDL value and triglyceride after getting positive blood culture to know the change in the value of both HDL and triglyceride during after initiation of antibiotics treatment and the results was:

1: if HDL value continues dropping for more than 5 days, it supposed to be due to failure of treatment due to different causes and patient was going to die

2: if HDL value started to elevate, it supposed to indicate that treatment is succeeded to cure the patient from septicemia and SIRS.

3: if HDL value elevated 5 scales (i.e. from 7 to 12) another blood culture was done to confirm if infection is cured or not .it was surprised that blood culture was negative and antibiotics treatment can be stopped. The type of bacteria that caused septicemia was different from patient to patient according to the laboratory reports (table: 2)

Table: 1 value of HDL and triglyceride at time of getting a positive blood culture, with time per day to get a positive blood culture. It is clear that all patient with HDL (< 5 mg/dl) and triglyceride (> 150 mg/dl), some of patients developed SIRS rapidly and some not .

Patients BSA%	HDL value (mg/dl)	Triglyceride (mg/dl)	Clinical status	Time to develop a +ve blood culture	Final state of patient
60%	<5	235	SIRS*	7	Died***
65%	9	170	SIRS	10	Lived***
60%	< 5****	163	Septicemia**	8	lived
80%	< 5	223	SIRS	7	died
90%	< 5	164	SIRS	15	died
65%	< 5	217	SIRS	11	died
52%	< 5	243	septicemia	17	lived
95%	13	151	SIRS	9	died
75%	12	213	SIRS	5	died
80%	12	435	Septicemia	8	died
35%	7	200	septicemia	11	lived
55%	7	180	septicemia	7	died
45%	< 5	213	SIRS	3	lived
55%	13	323	septicemia	6	died
65%	11	167	septicemia	20	died
60%	< 5	296	Septicemia	13	died
30%	< 5	261	septicemia	8	lived
35%	8	278	septicemia	8	lived
55%	12	154	septicemia	7	lived
90%	< 5	280	SIRS	4	died
30%	11	261	septicemia	6	lived
50%	9	180	septicemia	8	lived

*SIRS it is a systemic inflammatory response syndrome characterized by fever, tachycardia, tachypnea and other signs and symptoms in response to infection of bloodstream with germ.

**septicemia:it is also called poisoning of bloodstream, the infection of blood with bacteria, virus, parasite or fungi.

*** died or lived: is referred to the final state of the patients (some of patients are died and some of them are lived)

**** <5: it is the lowest value of HDL that can be getting by system ARCHITECT® C 4000, It means from (4 to 0) mg/dl.

Table: 2 Type of bacteria in blood culture with number of patients infected with that type of bacteria according to VITEK 2 system's reports.

Type of bacteria	Number of patients
Enterococcus Faecium	3
Klebsiella pneumonia	4
Pseudomonas aeruginosa	1
Pseudomonas luteola	1
Enterbacter capitis	2
E . coli	1
Pantoea agglomerans	2
Staph. aureus	2
Staph. Epidermidis	4
Citrobacter freundii	2

It was easy to conclude a close relationship between blood poisoning and HDL value in burnt patient.

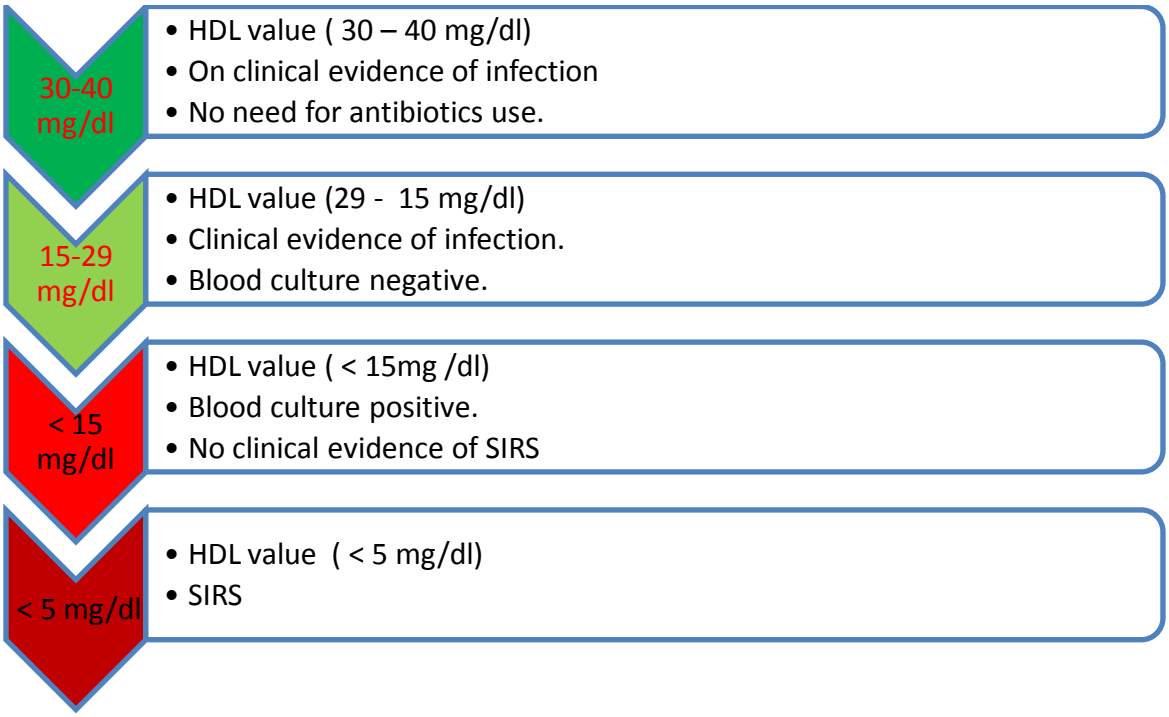

Figure: 1 this figure represents the clinical state of the burnt patient according to HDL value and thus can indicate the best time for initiation of antibiotics treatment.

During our study all patients with BSA% more than 20% had a HDL value less than normal range (40 – 60 mg/dl). In this study patient with HDL value from 40 – 30 mg/dl was free of any signs or symptoms of infection. While patients with HDL value (30- 15 mg/dl) were with fever, pain at burnt wound, and some with cellulitis with a negative blood culture. On the other side patients with HDL value (< 15 mg/dl) with a positive blood culture even if there was no clinical evidence of septicemia (figure:1). Finally patients with HDL value (< 5 mg/dl) were with SIRS.

CONCLUSION

HDL value can be used as a biomarker of septicemia, it is easy, cheap and the best septicemia biomarker due to the wide range of HDL normal value (40 – 60 mg/dl) compared with other biomarkers, also wide range from normal value to HDL value in septicemia (from 40 mg/dl to 15 mg/dl).

REFERENCES

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR: Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001, 29:1303-1310.
2. Hotchkiss RS, Karl IE: The pathophysiology and treatment of sepsis. N Engl J Med 2003, 348:138-150.
3. Gullo A, Bianco N, Berlot G: Management of severe sepsis and septic shock: challenges and recommendations. Crit Care Clin 2006, 22:489-501.
4. Lever A, Mackenzie I: Sepsis: definition, epidemiology, and diagnosis. BMJ 2007, 335: 879-883.
5. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M: Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006, 34:1589-1596.
6. Zambon M, Ceola M, Almeida-de-Castro R, Gullo A, Vincent JL: Implementation of the Surviving Sepsis Campaign guidelines for severe sepsis and septic shock: we could go faster. J Crit Care 2008, 23:455-460.
7. Biomarkers Definitions Working Group: Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001, 69:89-95.
8. Marshall JC, Reinhart K: Biomarkers of sepsis. Crit Care Med 2009, 37:2290-2298.
9. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, Reinhart K, Angus DC, Brun-Buisson C, Beale R, Calandra T, Dhainaut JF, Gerlach H, Harvey M, Marini JJ, Marshall J, Ranieri M, Ramsay G, Sevransky J, Thompson BT, Townsend S, Vender JS, Zimmerman JL, Vincent JL: Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008, 36:296-327.
10. Povoa P, Coelho L, Almeida E, Fernandes A, Mealha R, Moreira P, Sabino H: C-reactive protein as a marker of infection in critically ill patients. ClinMicrobiol Infect 2005, 11:

101-108.

11. Schmit X, Vincent JL: The time course of blood C-reactive protein concentrations in relation to the response to initial antimicrobial therapy in patients with sepsis. Infection 2008, 36:213-219.
12. Clyne B, Olshaker JS: The C-reactive protein. J Emerg Med 1999, 17:1019-1025.
13. Nakamura A, Wada H, Ikejiri M, Hatada T, Sakurai H, Matsushima Y, Nishioka J, Maruyama K, Isaji S, Takeda T, Nobori T: Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock 2009, 31:586-591.
14. Luzzani A, Polati E, Dorizzi R, Rungatscher A, Pavan R, Merlini A: Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003, 31:1737-1741.
15. Tang BM, Eslick GD, Craig JC, McLean AS: Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 2007, 7:210-217.
16. Giamarellos-Bourboulis EJ, Giannopoulou P, Grecka P, Voros D, Mandragos K, Giamarellou H: Should procalcitonin be introduced in the diagnostic criteria for the systemic inflammatory response syndrome and sepsis? J Crit Care 2004, 19:152-157.
17. D. L. Dragnov and B. N. La Du, “Pharmacogenetics of paraoxonases: a brief review,” Naunyn-Schmiedeberg's Archives of Pharmacology, 2004;369(1): 78–88,.
18. A. J. Luis, “Atherosclerosis,” Nature, vol. 2000; 407(6801): 233–241,.
19. D. M. Shih, L. Gu, Y. R. Xia et al., “Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis,” Nature, 1998; 394(6690): 284–287,.
20. M. Navab, G. M. Ananthramaiah, S. T. Reddy et al., “The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL,” Journal of Lipid Research, vol. 45, no. 6, pp. 993–1007, 2004.
21. .T. Vaisar, S. Pennathur, P. S. Green, et al., “Shotgun proteomics implicates protease inhibition and complement activation in the anti-inflammatory properties of HDL,” The Journal of Clinical Investigation, vol. 117, no. 3, pp. 746–756, 2007.
22. S. M. Gordon, D. Jingyuan, L. J. Lu, and W. S. Davidson, “Proteomic characterization of human plasma high density lipoprotein fractionated by gel filtration chromatography,” Journal of Proteome Research, vol. 9, no. 10, pp. 5239–5249, 2010.
23. A. Von Eckardstein, Y. Huang, and G. Assmann, “Physiological role and clinical relevance of high-density lipoprotein subclasses,” Current Opinion in Lipidology, 1994;

5(6): 404–416.

24. H. O. Mowri, W. Patsch, L. C. Smith, A. M. Gotto, and J. R. Patsch, "Different reactivities of high density lipoprotein2 subfractions with hepatic lipase," Journal of Lipid Research, 1992;33(9):1269–1279.
25. T. Miida, M. Kawano, C. J. Fielding, and P. E. Fielding, "Regulation of the concentration of preβhigh-density lipoprotein in normal plasma by cell membranes and lecithin-cholesterol acyl transferase activity," Biochemistry, 1992; 31(45): 11112–11117.
26. F. Gu, M. K. Jones, J. Chen et al., "Structures of discoidal high density lipoproteins: a combined computational-experimental approach," The Journal of Biological Chemistry, 2010;285(7): 4652–4665.
27. M. A. Connelly and D. L. Williams, "Scavenger receptor BI: a scavenger receptor with a mission to transport high density lipoprotein lipids," Current Opinion in Lipidology, 2004; 15(3): 287–295,
28. M. A. Kennedy, G. C. Barrera, K. Nakamura et al., "ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation," Cell Metabolism, 2005;1(2): 121–131.
29. R. S. Rosenson, H. B. Brewer Jr., M. J. Chapman, et al., "HDL measures, particle heterogeneity, proposed nomenclature, and relation to atherosclerotic cardio-vascular events," Clinical Chemistry, 2011;57, 3: 392–410.
30. L. Calabresi, M. Gomaraschi, and G. Franceschini, "Endothelial protection by high-density lipoproteins: from bench to bedside," Arteriosclerosis, Thrombosis, and Vascular Biology, 2003; 23(10): 1724–1731.
31. A. J. Murphy, J. P. F. Chin-Dusting, D. Sviridov, and K. J. Woollard, "The anti inflammatory effects of high density lipoproteins," Current Medicinal Chemistry, 2009; 16(6) : 667–675,.