Isfahan University of Medical Sciences
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Thesis Project For
M.Sc. Degree

Evaluation of CD11b and CD64 markers in Neonatal Sepsis for rapid diagnosis and compare with blood culture and serum CRP level.

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Summary:

Introduction: Neonatal sepsis is a life-threatening disease with an incidence of 1 to 10 per 1000 live births and a mortality rate of 15% to 50%. The clinical signs are nonspecific and indistinguishable from those caused by a variety of neonatal noninfective disorders. The aim of this study was to determine sensitivity, specificity, positive and negative predictive value of CD11b and CD64, two neutrophil markers, in early diagnosis of neonatal sepsis.

Methods: In this study were comprised 65 neonates with a gestational age of 27 to 38 weeks who suspected for sepsis within 28 days of life. 1 cc of whole blood was obtained from neonates to determine of CD11b and CD64 expression on peripheral blood neutrophils by flow cytometry. CRP was measurement qualitatively. Neonates were classified into two groups. Classification was based on positive blood culture. In the sepsis group (n = 8), all of neonates were positive blood culture and had clinical symptoms. In the suspected group (n = 57), each neonate had at least 1 clinical sign but negative blood culture. 12 healthy term neonates with physiologic hyperbilirubinemia classifed in control group.

Results: Neutrophil CD11b was elevated in sepsis and suspected group but this increase was not significant between three groups (P = 0.69). CD64 was elevated in sepsis group and this increase was significant (P<0.001). CRP in 4 neonates with sepsis was positive and other neonates were negative.

Sensitivity and specificity of CD11b and CD64 were 75%, 100%, 100% and 92.3% respectively. The negative and positive predictive value of CD11b and CD64 for identifying sepsis were 86%, 100%, 100% and 88% respectively.

Discussion: Sensitivity of CD64 was higher than CD11b in neonatal sepsis. Although, specificity of CD11b was higher than CD64. Combining the use of CD64 with CD11b further enhances the ability to diagnosis infection and improve the sensitivity and negative predictive value to 100%.

Key word: Neonatal sepsis, CRP, CD11b, CD64.

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2. **McKenney WM.** Neonatal Nursing: Understanding the Neonatal Immune System: High Risk for Infection. *Critical Care Nurse. 2001; Vol 21, No. 6: 35-47.*
3. **Buckley R.** Infections of the neonatal infant. In: **Behrman Re. Kliegman R. Jensen HB.** eds. Behrman: Nelson textbook of Pediatrics. 16th ed. Philadelphia, Pa: *WB Saunders CO; 2000: 538-543.*
4. **Lewis D.** Host defense mechanisms against bacteria, fungi, viruses and nonviral pathogens. In: **Polin RA. Fox WD. Fletcher J.** eds. Fetal and Neonatal Physiology. Vol 2.2nd ed. Philadelphia, Pa: *WB Saunders Co; 1998: 1869-1919.*
5. **Cole F.** Immunology. In**: Taeusch HW. Ballard RA. Avery Me.** eds. Schaeffer and Avery's Diseases of the newborn. 6th ed. Philadelphia, USA, Pa: *WB Saunders CO: 1991:305-320.*
6. **Lawton A.** B-cell development. In: Polin RA, Fox WB, **Fletcher J.** eds. Fetal and neonatal Physiology. Vol 2.2nd ed. Philadelphia, Pa; *WB Saunders Co: 1998: 1924-1930.*
7. **Pappas BE.** Primary immunodeficiency disorders in infancy. *Neonatal Netw.1999;18:13-22.*

8. **Lewis D. Wilson C.** Developmental immunology and role of host defenses in neonatal susceptibility to infection. In: **Remington JS. Klein JO**, eds. Infectious Diseases of the Newborn Infant. 4th ed. *Philadelphia, Pa: WB Saunders Co: 1995:20-98.*

9. **Witek-Janusek L. Cusack C.** Neonatal Sepsis: confronting the challenge. *Crit Care Nurs Clin North Am. 1994; 6:405-419.*

10. **Crockett M.** Physiology of the neonatal immune system. *J Obstet Gynecol Neonatal Nurse. 1995; 24: 627-634.*

11. **Speer C. Johnston RJr.** Neutrophil function in newborn infants. In: **polin RA, Fox WB, Fletcher J,** eds. Fetal and Neonatal Physiology. Vol 2.2nd ed. Philadelphia, Pa: *WB Saunders Co: 1998: 1954-1960.*

12. **Cole FS.** Bacterial Infections of the Newborn. In: **Taeusch HW. Ballard RA.** Avery's diseases of the newborn. 7th ed. Philadelphia: *WB Sunders Co. 1998: 490-512.*

13. **Avroy A. Fanaroff RY.** Neonatal- Perinatal Medicine: Diseases of the Fetus and Infant. 7th ed. Lowis: *Mosby; 2002: 860- 392.*

14. **Espinosa EL. Montes AT. Luis F. Gonzalez P. Baranda L.** Expression of CD64 as a potential marker of neonatal sepsis. *Pediatric Allergy and Immunology. 2002:13:319-327.*

15. **Panero A. Pacifico L. Rossi N. Mancuso G. Stegagno M. Chiesa C.** Interleukin-6 in neonates with early and late onset infection. *Pediatr Infect Dis J. 1997:14: 370-5.*

16. **Horns KM.** Neoteric Physiologic and Immunologic Methods for Assessing Early-Onset Neonatal Sepsis. *J Perinat Neonat Nurs. 2000:13(4):50-66.*

17. **Speer CH. Bruns A. Gahr M.** Sequential determination of CRP, α_1–antitrypsin and haptoglobin in neonatal septicaemia. *Acta Paediatr Scand. 1983;72: 679-83.*

18. **Howard MR. Smith RA.** Early diagnosis of septicaemia in preterm infants from examination of peripheral blood films. *Clin Lab Haematolo. 1999; 21: 365-8.*

19. **Da silva O. Ohlsson A. Kenyon C.** Accuracy of leukocyte indices and C-reactive protein for diagnosis of neonatal sepsis: *a critical review. Pediatr Infect Dis J. 1995; 14: 362-6.*

20. **Ng PC. Cheng SH. Chui KM.** Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecules, and C-reactive protein in preterm very low brithweight infants. *Arch Dis Child Fetal Neonatal Ed. 1997; 77: F221-7.*

21. **Berger C. Uehligner J. Ghelfi D.** Comparison of C-reactive protein and blood cell count with differential in neonates at risk of septicaemia. *Eur J Pediatr. 1995; 154: 138-44.*

22. **Jurges ES. Henderson DC.** Inflammatory and immunological markers in preterm infants: correlation with disease. *Clin Exp Immunol. 1996; 105: 551-5.*

23. **Volanakis JE.** Human C-Reactive Protein: expression, structure and function. *Review. Molecular Immunology. 2001; 38: 189-197.*

24. **Clyne B. Olshaker JS.** The C-Reactive Protein. *The Journal of Emergency Medicine. 1999; 17(6): 1019- 1025.*

25. **Mold C. Kingzette M. Gewurz H.** C-reactive protein inhibits activation of the alternative pathway by increasing the interaction between factor H and C3b. *J Immunol. 1984; 133: 882-885.*

26. **Mold C. Gewurz H. Du Clos TW.** Regulation of complement activation by C-reactive protein. *Immunopharmacology. 2001; 42: 23-30.*

27. **Stein MP. Mold C. Du Clos TW.** C-reactive protein binding to murine leukocytes requires Fc gamma receptors. *J. Immunol. 2000; 164: 1514-1520.*

28. **Mold C. Gresham HD. Du Clos TW.** Serum amyloid P component and C-reactive protein mediate phagocytosis through murine FcγRs. *J. Immunol. 2001; 166: 1200-1205.*

29. **Edwards KM. Gewurz H. Lint TF. Mold C.** A role for C-reactive protein in the complement-mediated stimulation of human neutrophils by type 27 streptococcus pneumoniae. *J. Immunol. 1982; 128: 2493-2496.*

30. **Rohde LE. Hennekens CH. Ridder PM.** Survey of C-reactive protein and cardiovascular risk factors in apparently healthy men. *Am. J. Cardiol. 1999; 84: 1018-1022.*

31. **Ridker PM. Hennekens CH. Buring JE. Rifai N.** C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl. J. Med. 2000; 342: 836-843.*

32. **Simms HH. D' Amico R.** Lipopolysaccharide induces introcytoplasmic migration of the polymorphonuclear leukocyte CD11b/CD18 receptor. *Shock. 1995; 3: 196-203.*

33. **Ng PC. Li K. Wong RPO.** Neutrophil CD64 expression: a sensitive diagnistic marker for late-onset nosocomial infection in very low brithweight infants. *Pediatr Res. 2002;51: 296-303.*

34. **Balsam LB. Liang TW. Parkos CA.** Functional Mapping of CD11b/CD18 Epitopes Important in Neutrophil-Epithelial Interactions: A Central Role of the I Domain. *The Journal of Immunology. 1998; 160: 5058-5065.*

35. **Oxvig C. Springer TA.** Experimental support for a beta-propeller domain in integrin alpha-subunits and a calcium binding site on its lower surface. *Proc Natl Acad Sci USA. 1998; 95: 4870-5.*

36. **Hynes RO.** Integrins: bi-directional, allosteric signaling machines. *Cell. 2002; 110: 673-87.*

37. **Hevia JA. Arenas AG. Barrera D. Velazquez MV.** Gram-negative bacteria and phagocytic cell interaction mediated by complement receptor 3. *FEMS Immunology and Medical Microbiology. 2002;34: 255-266.*

38. **Velazquez MAV. Barrera D. Arenas AG. Rosales C. Hevia JA.** Macrophage- Mycobacterium tuverculosis interactions: role of complement receptor 3. *Microbial Pathogenesis. 2003; 35: 125-131.*

39. **Pol WI. Winkel JGJ.** IgG receptor polymorphisms: risk factor for disease. *Immunogenetics. Review. 1998; 48: 222-232.*

40. **Heijnen IAFM. Vuget MJ. Fanger NA. Graziano RF.** Antigen targeting to myeloid-specific FcγRI/CD64 triggers enhanced antibody responses in transgenic mice. *J Clin Invest. 1996; 97: 331-338.*

41. **Liu C. Goldstein J. Graziano RF. He J.** FcγRI-targeted fusion proteins result in efficient presentation by human monocytes of antigenic and antagonist T cell epitopes. *J Clin Invest. 1996; 98: 2001-2007.*

42. WWW. bioteach. ubc. ca/Molecular Biology/FlowCytometry/

43. **Weave JL.** Introduction to Flow Cytometry. *Methods. 2000; 21(3): 199-201.*

44. **Weirich E. Rabin RL. Maldonado Y. Benitz W.** Neutrophil CD11b expression as a diagnostic marker for early-onset neonatal infection. *J Pediatr. 1998; 132: 445-51.*

45. **Nupponen I. Andersson S. and et al.** Neutrophil CD11b expression and circulating Interleukin-8 as diagnostic markers for early-onset neonatal sepsis. *Pediatrics. 2001; 108(1): 1-6.*

46. **Cui YB. Du LZ. Chen YZ. Yu YB. Wang FM.** Expression of neutrophil adhesion molecule CD11b as an early diagnosis for neonatal sepsis. Abstract. *Zhonghua Er Ke Za Zhi. 2003; 41(5): 348-51.*

47. **Weinschenk NP. Farina A. Bianchi DW.** Premature infants respond to early-onset and late onset sepsis with leukocyte activation. *J Pediatr 2000; 137: 345-50.*

48. **Fjaertoft G. Hakansson L. Edward U. Foucard T. Venge P.** Neutrophils from term and preterm newborn infants express the high affinity Fcγ-receptor I (CD64) during bacterial infection. *Pediatr Res. 1999; 45: 871-876.*

49. **Lehr HA. Krombach F. Munzing S. bodlaj R. Glaubitt Si. Seiffge D. hubner C. et al.** in vitro effects of oxidized low density lipoprotein on CD11b/CD18 and L-selectin presentation on neutrophils and monocytes with relevance for the in vivo. Situation. *Am J Pathol. 1995; 146: 218-227.*

50. **Simms HH. D'Amico R.** Lipopolysaccharide induces intracytoplasmic migration of the polymorphonuclear leukocyte CD11b/CD18 receptor. *Shok. 1995; 3: 196-203.*

51. **de Hass M. Vossebeld PJM. Von dem Borne AEGK. Roos D.** Fcγ receptors of phagocytes. *J Lab Clin Med. 1995; 126: 330-341.*

52. **Sanchez-Mejorada G. Rosales C.** signal transduction by immunoglobulin Fc receptors. *J leukoc Biol. 1998; 63: 521-533.*

53. **Ng PC. Li G. Chui KM. Chu WCW. Li K. Wong RPO. Chik KW. Wong E. Fok TF.** Neutrophil CD64 is a sensitive diagnostic markers for Early-onset neonatal infection. *Pediatr Res. 2004; 56(5): 796-803.*

54. **Ng PC.** Diagnistic markers of infection in neonates. *Review. Arch Dis Child Fetal Neonatal Edu. 2004; 89: 229- 235.*