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Measurement of Circulating Cytokines and Immune-Activation Markers by Multiplex Technology in the Clinical Setting: What Are We Really Measuring?

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Abstract

Measurement of circulating cytokine levels can provide important information in the study of the pathogenesis of disease. John L. Fahey was a pioneer in the measurement of circulating cytokines and immune-activation markers and a leader in the quality assessment/control of assays for measurement of circulating cytokines. Insights into the measurement of circulating cytokines, including consideration of multiplex assays, are presented here.

Keywords

cytokine; immunology; multiplex assay

I. PERSPECTIVES

Immune and nonimmune cells secrete cytokines that change the conduct and properties of target cells and can function as a network or cascade of complex interactions and communications among cells. Cytokines induce their biological effects at minute pharmacological doses and have short half-lives.¹ Cytokine variations are observed after tissue injury (trauma, surgery, or radiation), acute and chronic infection, sepsis, autoimmune disease, cancer, aging, and other disorders.

Cytokines appearing in the blood are those not consumed at local sites or not rapidly removed from circulation. In general, the levels of many cytokines circulating in the blood of either healthy or diseased individuals are below the limit of detection by commercially available assays kits. The cytokine blood levels that can be measured may also show substantial variability because the values in the assays are at or near their limits of precise measurement.² In addition, blood levels of cytokines are susceptible to preanalytical factors such as patient behaviors, fasting, drugs, diurnal rhythms, physical activity, blood collection, handling, processing, and storage, as well as analytical factors such as assay methods, reagents and references standards of the assay, and calibrated standards against the World Health Organization (WHO) reference used in the kit.

Recently, advances in laboratory technologies including flow cytometrics, Luminex bead-based assays, Meso Scale Discovery assays, planar multiplex assays, and procedural improvements such as selection of high-quality reagents, preference of a highly sensitive

assessment format, and usage of a smaller specimen volume have made it possible to measure a more comprehensive panel of cytokines in serum/plasma within a short period of time.

The accuracy and precision of these multiplex assays with novel technologies and platforms depend on the selection of a specific high-quality pair of capture and detection antibodies of the cytokine of interest, operator experience, standardization of multiplex cytokine measurement, and performance of the assay under restricted in-house quality control and well-established national quality assurance programs.

John L. Fahey was a pioneer in the measurement of cytokines and biomarkers of immune activation. His group was the first to document that human immunodeficiency virus (HIV) could induce interleukin (IL)-6 production and secretion³ and that viral load was associated with serum levels of tumor necrosis factor- α and other immune activation biomarkers.⁴ In addition, more than 25 years ago, he identified that measurement of serum levels of immune-activation markers such as β 2 microglobulin, neopterin, soluble IL-2Ra, immunoglobulin A (IgA), and HIV P24, in conjunction with measurement of CD4⁺ T cells, can provide valuable information for understanding the pathogenesis, prognosis, and treatment of HIV-1 infection and AIDS.⁵ He also realized that these potent soluble mediators of immune function and regulation would be disrupted in various disease states and that their measurements would provide profound insights into the pathogenesis of many immune system disorders.

Fahey was also a leader in the measurement of cytokines in in vivo specimens as well as in quality assurance and quality control of assays used to measure these bioactive molecules. In laboratory quality control, good laboratory practice (GLP) must be followed for checking the validity of the cytokine and soluble marker assay results either by ELISA or multiplex, just as national well-established quality assurance programs should be followed to enhance laboratory performance, monitoring, reporting, record keeping, and archiving. Great progress has been made in this area and a procedure recommended for in-house quality control of cytokines and soluble marker testing in body biological fluids was outlined and published in 1998.⁶ Fahey and coworkers led the first group to develop an external evaluation program for measuring the performance of laboratories that were testing cytokines, chemokines, and immune activation markers in biological fluids for the Adults and Pediatric AIDS Clinical Trials Groups. The group also reported that the potential value of the quality assurance program on a larger, conceivably national, scale would help physicians in the evaluation of many autoimmune diseases, infectious diseases affecting the immune system, and other disorders characterized by a changing balance within the immune system.⁷

In 2010, the National Institutes of Health and the National Institute of Allergy and Infectious Diseases (NIH/NIAID) and the Cancer Immunotherapy Consortium of the Cancer Research Institute collaborated to develop and implement a Luminex assay-proficiency-testing program as part of the NIH/NIAID-sponsored External Quality Assurance Program Oversight Laboratory (EQAPOL) at Duke University. The program currently monitors 25 domestic and international sites with two external proficiency panels per year. Each panel

includes a deidentified commercial Luminex assay kit with standards to quantify human interferon- γ , tumor necrosis factor- α , IL-6, IL-10, and IL-2 in addition to a series of recombinant cytokine-spiked human serum samples.⁸

Although the multiplex platforms and ELISA-based systems exhibit distinct strengths and weaknesses, the accurate measurement of these cytokines may provide reliable information regarding disease diagnosis, staging, prognosis, and evaluation of therapy. Several studies have compared cytokine levels determined by ELISA and multiplex immunoassays, with results showing both good and poor correlations between the methods and overestimation of cytokine levels.^{9–13} Therefore, despite identical preanalytical sample condition throughout collection, separation, and storage, observation of cytokine-measurement data variability due to the use of kits from different manufacturers was not surprising.¹⁴

Thus, this question can be asked: What are we really measuring? All of the advancements in the knowledge of cytokine action, evolved laboratory technologies, and improvement in rapid cytokine measurement turnaround time did not resolve the implementation of these novel biomarkers into clinical practice. This proves to be a long and challenging process that includes convincing physicians of the usefulness of cytokine measurements.¹⁵ Therefore, multiplex assays for cytokines have not met the requirements of a clinical test and are used only for investigational purposes. In addition, they are not subject to CLIA.

In most cases, research testing for which patient-specific results are reported from the laboratory and wherein those results will be or could be used “for the diagnosis, prevention, or treatment of any disease or impairment of, or the assessment of the health of human beings” is presumed to be subject to CLIA, absent evidence to the contrary. In cases wherein patient-specific test results are maintained by a statistical research center for possible use by investigators and the results are not reported as patient specific, such testing would not fall under CLIA regulations.¹⁶

In summary, the multiplex cytokines assay based on the CLIA definition has only statistical research value and is a less robust test for serum and plasma in the clinical setting when compared carefully side by side with conventional ELISA. Abundant circulating blood proteins such as α -2 macroglobulin, autoantibodies, assay matrices, cross-reaction of multiple antibodies used in the multiplex format, and lack of assay standardization can greatly impact the reliability and reproducibility of these assays. Those factors may affect multiplex assays more than they impact conventional ELISA. Accurate measurement of blood cytokines by a multiplex platform with all its advantages is still problematic, and the clinical usefulness of cytokine levels in serum or plasma by the multiplex assay continues to remain uncertain.

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ABBREVIATIONS

AIDS	acquired immune deficiency syndrome
CLIA	Clinical Laboratory Improvement Amendments
ELISA	enzyme-linked immunosorbent assay

References

- Whiteside TL. Cytokine assays. *Biotechniques*. 2002; 33:S4–S15.
- Fahey JL. Cytokines, plasma immune activation markers, and clinically relevant surrogate markers in human immunodeficiency virus infection. *Clin Diagn Lab Immunol*. 1998; 5:597–603. [PubMed: 9729522]
- Nakajima K, Martínez-Maza O, Hirano T, Breen EC, Nishanian PG, Salazar-Gonzalez JF, Fahey JL, Kishimoto T. Induction of IL-6 (B cell stimulatory factor-2/IFN- β 2) production by HIV. *J Immunol*. 1989; 142:531–36. [PubMed: 2783441]
- Salazar-Gonzalez JF, Martinez-Maza O, Aziz N, Kolberg JA, Yeghiazarian T, Shen LP, Fahey JL. Relationship of plasma HIV-RNA levels and levels of TNF- α and immune activation products in HIV infection. *Clin Immunol Immunopathol*. 1997; 84:36–45. [PubMed: 9191882]
- Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, Giorgi JV. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. *New Engl J Med*. 1990; 322(3):166–72. [PubMed: 1967191]
- Aziz N, Nishanian P, Fahey JL. Levels of cytokines and immune activation markers in plasma in human immunodeficiency virus infection: Quality control procedures. *Clin Diagn Lab Immunol*. 1998; 5:755–61. [PubMed: 9801330]
- Fahey JL, Aziz N, Spritzler J, Plaeger S, Nishanian P, Lathey JL, Seigel J, Landay AL, Kilarui R, Schmitz JL, White C, Wara DW, Akridge R, Cutili J, Douglas SD, Reuben J, Shearer WT, Nokta M, Pollard R, Schooley R, Asthana D, Mizrahi Y, Waxdal M. Need for an external proficiency testing program for cytokines, chemokines, and plasma markers of immune activation. *Clin Diagn Lab Immunol*. 2000; 7:540–8. [PubMed: 10882648]
- Lynch HE, Sanchez AM, D'Souza MP, Rountree W, Denny TN, Kalos M, Sempowski GD. Development and implementation of a proficiency testing program for Luminex bead-based cytokine assays. *J Immunol Methods*. 2014; 409:62–71. [PubMed: 24801479]
- Breen EC, Reynolds SM, Cox C, Jacobson LP, Magpantay L, Mulder CB, Dibben O, Margolick JB, Bream JH, Sambrano E, Martínez-Maza O, Sinclair E, Borrow P, Landay AL, Rinaldo CR, Norris PJ. Multisite comparison of high-sensitivity multiplex cytokine assays. *Clin Vaccine Immunol*. 2011; 18:1229–42. [PubMed: 21697338]
- Dupuy AM, Kuster N, Lizard G, Ragot K, Lehmann S, Gallix B, Cristol JP. Performance evaluation of human cytokines profiles obtained by various multiplexed-based technologies underlines a need for standardization. *Clin Chem Lab Med*. 2013; 51:1385–93. [PubMed: 23314551]
- Churchman SM, Geiler J, Parmar R, Horner EA, Church LD, Emery P, Buch MH, McDermott MF, Ponchel F. Multiplexing immunoassays for cytokine detection in the serum of patients with rheumatoid arthritis: Lack of sensitivity and interference by rheumatoid factor. *Clin Exp Rheumatol*. 2012; 30:534–42. [PubMed: 22704737]
- Prabhakar U, Eirikis E, Miller BE, Davis HM. Multiplexed cytokine sandwich immunoassays: Clinical applications. *Methods Mol Med*. 2005; 14:223–32.
- Elshal MF, McCoy JP. Multiplex bead array assay: performance evaluation and comparison of sensitivity to ELISA. *Methods*. 2006; 38:317–23. [PubMed: 16481199]
- Payette C, Blackburn P, Lamarche B, Tremblay A, Bergeron J, Lemieux I, Després JP, Couillard C. Sex differences in postprandial plasma tumor necrosis factor- α , interleukin-6, and C-reactive protein concentrations. *Metabolism*. 2009; 58:1593–601. [PubMed: 19604525]

15. Burska A, Boissinot M, Ponchel F. Cytokines as biomarkers in rheumatoid arthritis. *Mediat Inflamm.* 2014; 2014:545493.
16. Clinical Laboratory Improvement Amendments of 1988 (CLIA) (57 FR 7139, Sec. 493.1). <http://wwwn.cdc.gov/clia/>