Published in final edited form as:

Methods. 2013 May 15; 61(1): 1–2. doi:10.1016/j.ymeth.2013.05.019.

Editorial for “Cytokine Methods”

Daniel Remick, M.D. and

Boston University, remickd@bu.edu

James Duffy

Northeastern University, School of Law, jsduffy42@gmail.com

Cytokines are small proteins which make up a portion of the innate immune system. They are secreted by several different cells in response to multiple different stimuli. Cytokines are critical components of the immune system and govern numerous aspects of inflammation. These proteins are such critical components in health and disease that multiple drugs have been created specifically targeting different cytokines. Perhaps the best known of these are the inhibitors of tumor necrosis factor (TNF) which have been successfully used to treat chronic inflammatory conditions such as rheumatoid arthritis and inflammatory bowel disease. Given their prominent roles in regulating inflammation, interest in their accurate measurement helps both guide therapy and understand their mechanisms of action.

A special issue of *Methods* devoted to the measurement of cytokines was published in 2006 [1]. Articles in the first issue have been widely cited including several that have been referenced more than a dozen times [2–4]. A comparison of the contents of these two issues shows both changes reflecting advances in the field and the consistency of the methods used for cytokine analysis. In terms of consistency, antibody based assays are the cornerstone of virtually all cytokine detection systems. In fact, there are virtually no current publications that use biological assays for the detection or quantification of cytokines. This move from biological assays to immunoassays was already well underway by 2006. The near complete absence of bioassays reflects the ease of use for immunoassays coupled with better antibodies and better detection systems. Flow cytometry for intracellular detection of cytokines has also rapidly evolved from state-of-the-art to a routine laboratory technique for many investigators.

This issue is organized into discrete sections. The first section covers basic concepts of cytokine measurements. The first paper is a timely review of whether measuring cytokines has value in patients with trauma or sepsis (Moldawer paper, 10.1016/j.ymeth.2013.04.024). Both of these diseases are examples of relatively acute immune reactions which evolve over a few days, which contrasts with the use of cytokine inhibitors in chronic diseases. This paper highlights how cytokine measurements may be useful as biomarkers for the disease process and discusses reasons behind the limitations of cytokine measurements. Cytokines may be measured in multiple different types of samples including plasma, serum, dried blood spots or tissue biopsies. Several important sample handling parameters need to be

followed in order to provide consistent results. Keustermans et al. review many of these issues in their submission (METHODS-D-12-00188). Proper handling and processing at the very start of a study is important so that downstream measurements are accurate. This may be especially true when samples have been stored for prolonged periods.

Even if the samples have been optimally collected and processed there are pitfalls which may make cytokine measurements inaccurate. As already mentioned, virtually all cytokine measurements are currently performed with an immunological assay. Bartels and Ribel-Madsen discuss how the presence of heterophile antibodies or human anti-animal antibodies may interfere with cytokine measurements (METHODS-D-12-00097). These substances will interfere with either single cytokine measurements or multiplex cytokine assays. Many commercial companies now market multiplex cytokine kits which will measure more than a dozen cytokines in a single assay. These assays have become very popular and may actually decrease the total cost of running an experiment, since a small sample size may be sufficient. The utility and reliability of several of these assays are detailed in the manuscript by Tighe et al. (METHODS-D-12-00123).

Newer methods for measuring cytokines are covered in the next section. While the multiplex assays quantify several cytokines they cannot provide information about which cells make the cytokines. Intracellular cytokine staining accomplishes the goal of detecting the cells that make the cytokines. With appropriate techniques it is also possible to measure several cytokines in the same cell as described by Freer and Rindi (METHODS-D-12-00102). This flow cytometric technique can also determine cell viability and proliferation. Newer methods for measuring cytokines are provided in the paper by La Belle et al. (METHODS-D-12-00122) using conjugated nanoparticle-antibodies.

A significant advance in cytokine measurements has been the development of Interferon Gamma Release Assays (IGRAs). These assays are used to diagnose latent tuberculosis infections in patients and have been approved by government agencies for diagnostic testing. Whitworth et al. provide a review of the basic assay and clinical applicability (METHODS-D-12-00128). Another novel use of cytokine assays is described in the paper by Portugal-Cohen and Kohen (METHODS-D-12-00103). In this technique, non-invasive sampling of skin secretions are sampled and used to measure cytokines. The technique may be helpful in following different diseases such as psoriasis.

The final section is devoted to a techniques for analysis of the cytokine data. Whenever measurements are made in biological samples there is a possibility of assay error. While not all errors can be corrected, Natarajan and Remick describe a mathematical approach to determining cytokine concentrations when the standard curve has failed (METHODS-D-12-00108). Given the complexity of cytokine biology it is not surprising that analysis of the data has become more complex, requiring robust methods of analysis. A review of the principles that may be used for predictive modeling of complex data are presented in the paper by Spratt et al. (10.1016/j.ymeth.2013.01.002). This paper is provided as an overview so that investigators become aware of the method and is not meant to be an exhaustive tutorial.

Acknowledgments

The editors need to thank all of the authors for their contributions to this issue. Hopefully it will serve as a resource for methods to measure cytokines.

References

- [1]. Remick DG, Granger JI, Methods 38 (2006) 235–236. PMID. [PubMed: 16531067]
- [2]. de Jager W, Rijkers GT, Methods 38 (2006) 294–303. PMID. [PubMed: 16481197]
- [3]. Cox JH, Ferrari G, Janetzki S, Methods 38 (2006) 274–282. PMID. [PubMed: 16473524]
- [4]. Elshal MF, McCoy JP, Methods 38 (2006) 317–323. PMID: . [PubMed: 16481199]