Applications of Mass Cytometry in Clinical Medicine

The Promise and Perils of Clinical CyTOF

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KEYWORDS

• Mass cytometry • CyTOF • Flow cytometry • Minimal residual disease
• Acute leukemia • Aberrant marker expression

KEY POINTS

- Mass cytometry enables cytometric measurement of up to 50 parameters per single cell through the use of time-of-flight mass spectrometry and heavy-metal tagged antibodies.
- The high number of parameters facilitates analysis of highly complex cell populations and could be extremely useful for the diagnosis of malignant hematologic disorders, monitoring of minimal residual disease, and selection of treatment modalities.
- Mass cytometry could also be very useful for characterization of autoimmune disorders and monitoring of immunotherapy approaches.
- Several unique challenges associated with mass cytometry analysis need to be addressed before the technology could be used for clinical testing.
- The large amounts of data generated from mass cytometry assays are best analyzed using bioinformatic algorithms that enable a range of new approaches to the analysis of clinical samples.

INTRODUCTION

Flow cytometry has become an increasingly important diagnostic tool in the clinical laboratory for the study of malignancies, infectious diseases, and immune system function. Its utility stems from its ability to analyze single cells for their expression of virtually any protein to which an antibody, or similar reagent, can be specifically bound. This enables the identification and quantification of different cell types in complex biologic samples as well as the simultaneous assessment of a wide variety of functional properties of each cell. These many advantages have led flow cytometry to become

The author has nothing to disclose.
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Clin Lab Med 37 (2017) 945–964
http://dx.doi.org/10.1016/j.cll.2017.07.010 **labmed.theclinics.com**

a critical tool for the diagnosis of hundreds of medical disorders, and it is now routinely used for making critical treatment decisions. Despite its tremendous utility, however, most current clinical flow cytometry technologies are limited to analysis of 4 to 12 simultaneous measurement parameters. The dependence on fluorescent reporter molecules also makes flow cytometry susceptible to various artifacts due to the fluorescent properties of the cells being studied and interactions between the reagents being used to study them. Correcting for these artifacts requires extensive controls and experienced operators. Fortunately, the technology of cytometry continues to advance rapidly with newer machines and newer reagents being developed to improve upon these limitations. Perhaps none of these innovations is more transformative than the recent development of mass cytometry. Although there are now numerous publications using this technology and many excellent reviews,[1–4] this review focuses on its potential use in the clinical cytometry laboratory, where it may soon arrive and become an important tool to enhance cytometry's role in clinical practice.

Mass cytometry is a cytometric technique similar to fluorescent flow cytometry that detects antibody binding to cellular antigens through the use of mass spectrometry rather than fluorescent detection.[1,5] The technology uses binding of the same antigen-specific antibodies used in conventional flow cytometry, but measures this binding by attaching isotopically purified heavy metal atoms to the antibodies instead of fluorophores. The presence of the bound antibodies is then detected through the use of inductively coupled plasma ionization and time-of-flight mass spectrometry (ICP-MS TOF) analysis of the metal ions that were attached to each antibody. The advantage of this approach stems from the ability of ICP-MS to distinguish ions of different atomic weight with less than 1% signal spillover between adjacent masses. When done properly, mass cytometry data are nearly identical to fluorescent flow cytometry data for most antigens, and currently up to 40 to 50 parameters can be simultaneously recorded per cell without significant compensation or background from autofluorescence. Theoretically, the technology could measure up to 120 simultaneous parameters. This huge increase in parameters enables a broad range of new applications, all of which have the capacity to analyze highly complex cell populations in ways that were previously extremely difficult or impossible. The goal of this review is to provide a broad overview of the mass cytometry technology and how it might be applied in clinical settings. Specifically, the author addresses the major strengths of mass cytometry analysis, some limitations that will be particularly relevant for clinical applications, and some of the most common analysis methodologies used to explore the resulting data.

MASS CYTOMETRY BASICS

Mass cytometry is essentially a fusion of flow cytometry and ICP-MS, with the sample collection and processing steps being nearly identical to a flow cytometry workflow, and the data acquisition almost entirely based on ICP-MS TOF. The basic workings of a mass cytometer have been described in numerous publications,[1,5,6] so they will only be briefly summarized here. A schematic diagram of mass cytometry analysis (from Di Palma and Bodenmiller[4]) is shown in **Fig. 1**. Cells are first collected and processed in the same manner as a regular flow cytometry experiment. In general, the same cell processing methods used to assay the antigens of interest by fluorescent flow cytometry should be used for mass cytometry analysis of those same antigens. The cells are incubated (or "stained") with antibodies just as in standard flow cytometry assays (with the exception that the antibodies are conjugated to heavy metals rather than fluorophores). In most cases, the same antibody clones and protocols