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The use of multiplex platforms for absolute and relative protein quantification of clinical material

Lisa Christiansson[a], *Satu Mustjoki*[b], *Bengt Simonsson*[c], *Ulla Olsson-Strömberg*[c,d], *Angelica S.I. Loskog*[a], *Sara M. Mangsbo*[a,*]

[a] *Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Dag Hammarskjölds väg 20, 75185 Uppsala, Sweden*

[b] *Hematology Research Unit Helsinki, Department of Medicine, Division of Hematology, University of Helsinki and Helsinki University Central Hospital, Box 700, 00029 HUCH Helsinki, Finland*

[c] *Department of Medical Sciences, Uppsala University, Uppsala, Sweden*

[d] *Section of Hematology, Uppsala University Hospital, Uppsala, Sweden*

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ABSTRACT

When introducing multiplex platforms to measure protein content in precious clinical material there is an increased risk of cross reactivity, loss of sensitivity as well as accuracy. In this paper, four multiplex platforms and one singleplex platform were compared by running pre- and post-treatment plasma samples from CML patients. We found a variation of absolute protein concentrations between platforms. For some of the analytes and platforms, relative differences between pre- and post-treatment samples correlated. We conclude that absolute concentrations measured by different platforms should be compared with caution and comparing relative differences could be more accurate.

Significance

Today many research groups select a multiplex platform for their clinical research investigations based on the current availability of a certain machine in-house. Clinical material is usually scars and can rarely be used to explore intra- or inter assay variations with regard to absolute or relative protein concentrations; still the material as well as the assay can affect the results obtained. Herein we had an unique possibility to obtain and analyze clinical material from CML patients pre- and post TKI therapy to assess inflammatory patterns using a plethora of assays in order to investigate how absolute and relative protein concentrations correlate between these platforms.

1. Introduction

The possibility of multiplex protein analysis in complex samples such as human plasma or serum is becoming increasingly important and the multiplexed platforms are used in for example clinical diagnostics; biomarker validation; measurement of changes in protein abundance and modeling

* *Corresponding author at*: Uppsala University, Dag Hammarskjölds väg 20, 75185 Uppsala, Sweden. Tel.: +46 186119181.
E-mail address: sara.mangsbo@igp.uu.se (S.M. Mangsbo).

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networks, and measurement of pathways in physiological and disease states [1]. Multiplex protein quantification sets new and high demands on specificity of the detection reagents as well as buffers and other reagents used, as various targets may require different conditions for optimal quantification [2]. Consequently, multiplex assays require high levels of validation to ensure that the targets are detected optimally and with high specificity. Vignali has defined seven key parameters for creating a perfect multiplexed assay including specificity, sensitivity, simplicity, reliability, multiplexing abilities, cost and time [3].

Dependent on the application of the assay, some of these parameters may be more important than others. Additionally, certain patient material may affect assay results, requiring both the platform producer and the user to carefully evaluate data output with respect to the selected material. In this paper, we have investigated four different multiplex platforms for protein measurements and compared the results to results obtained from the golden standard of soluble protein quantification, the ELISA. Three of the multiplex platforms investigated (BioPlex, Meso Scale Discovery and Myriad RBM) as well as the ELISAs determine the absolute concentration of protein in the samples, while one platform (Somalogic) is designed as a discovery platform and measures only relative concentrations of protein.

The three multiplex platforms used for quantitative determination of proteins are, similar to ELISA, immunoassays where analytes are "sandwiched" between a capture- and a detection antibody before detection [2]. In a common singleplex ELISA the detection antibody is usually conjugated to an enzyme, that after the addition of enzyme substrate catalyses a reaction leading to color development in the microtiter plate. The intensity of the color is measured by spectrophotometry and corresponds to the amount of the specific protein to be detected in the unknown sample [4]. Another method, developed by Meso Scale Discovery, applies electrochemiluminescence to quantify the proteins in a microtiter plate [5]. This assay can be multiplexed since capture antibodies specific for different targets can be bound to distinct spots in the bottom of a microtiter plate. In the microsphere-based technology developed by Luminex Corporation, the Fc-parts of the capture antibodies are bound to groups of fluorescent microspheres. Each group of microspheres has slightly different fluorescence intensity and is covered with antibodies recognizing a distinct protein. The detection antibody is coupled to a fluorescent molecule to enable detection [2]. With the Luminex method two systems to quantify the level of protein are available. One of the systems, applied by Myriad RBM in this paper, is flow-based [6]. The other microsphere-based system utilizes magnetic fluorescent beads and is used by the BioPlex kit in this paper [7]. The methods described in this paper have different abilities of multiplexing, Meso Scale Discovery kits are available with up to 10 different analytes [8] while the Luminex technology can assay up to 50 analytes with the magnetic beads and up to 500 analytes with the flow technology [9].

Somalogic has developed a multiplex method for relative protein quantification of up to over 1100 analytes in one sample [10]. This technique is based on aptamer binding. Aptamers are folded, single-stranded, anionic oligonucleotides that can bind proteins with high specificity and affinity. Somalogic has developed Slow Off-rate Modified Aptamers called SOMAmers, these are modified aptamers that have a slower dissociation rate of the aptamer from its target protein compared to normal aptamers [11].

In this study, characteristics of different multiplex protein detection platforms and their ability to detect various proteins were compared. Plasma from patients with chronic myeloid leukemia (CML) before and after tyrosine kinase inhibitor (TKI) treatment was investigated in the study. We show that the absolute protein concentration varied when measured by the different platforms. However, fold changes in protein concentration after TKI treatment correlated for certain, but not all, analytes on some platforms.

2. Materials and methods

2.1. *Patient samples, control sample and sample preparation*

Frozen acid citrate plasma samples from patients with CML at baseline (before treatment) and after three months of treatment with imatinib (Gleevec, Novartis Pharmaceuticals, Basel, Switzerland) or dasatinib (Spyrcel, Bristol-Myers Squibb, New York, USA) were obtained from Helsinki University Central Hospital. The study was conducted in accordance with the Helsinki Declaration and was approved by the Regional Research Ethics Committee. All patients gave their written informed consent. Frozen samples were shipped to Uppsala, thawed on ice and subsequently vortexed. Aliquots of appropriate volumes for each analysis were pipetted into different tubes and refrozen at −70 °C. In each run a control sample containing chicken plasma was included.

2.2. *BioPlex Pro Human Cytokine 27-plex Panel*

Frozen plasma samples were thawed in 37 °C water bath and put on ice directly after thawing. Samples were centrifuged at 10,000 × *g* for 10 min prior to analysis to remove cell debris and aggregates. BioPlex Pro Human Cytokine 27-plex Panel (Bio-Rad Laboratories, Hercules CA, USA) analysis with MagPlex beads was performed in a flat bottom microtiter plate according to the manufacture's instructions. Briefly, samples were diluted 1:4 in sample diluent. Standard was reconstituted and diluted in a fourfold dilution series. Antibody coupled capture beads were prepared and plated. The bead solution was vortexed before addition to each well. Plate was washed, all wash steps were performed manually. First, wash solution was added to the plate that was subsequently covered with sealing tape. The plate was incubated on a shaker for 30 s at 1100 rpm and then for 1.5 min at 300 rpm. The plate was taken off the shaker and was incubated on a magnet for 1 min before the supernatant was discarded. After washing, diluted samples and standards were added in duplicates to the beads in the wells. The plate was incubated on a shaker and after incubation and wash, detection antibodies were added to each well. The plate was again incubated on a shaker and after another washing step, streptavidin-phycoerythin solution was added to the wells. After a last incubation step, beads