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How to series

How to: Measuring blood cytokines in biological psychiatry using commercially available multiplex immunoassays

Raoul Belzeaux[a,b,c], Marie-Noëlle Lefebvre[d], Anne Lazzari[e], Tifenn Le Carpentier[f,g], Julia-Lou Consoloni[a,b,c], Xavier Zendjidjian[b], Mocrane Abbar[h], Philippe Courtet[c,i], Jean Naudin[b], José Boucraut[a,c,j], Pierre Gressens[f,g], Nicolas Glaichenhaus[e], El Chérif Ibrahim[a,c,*]

[a] Aix-Marseille Univ., CNRS, CRN2M-UMR7286, Marseille, France
[b] Pôle de Psychiatrie, Assistance Publique-Hôpitaux de Marseille, Marseille, France
[c] Fondation FondaMental, Fondation de Recherche et de Soins en Santé Mentale, Créteil, France
[d] CIC-CPCET, AP-HM, Hôpital La Timone, Marseille, France
[e] Institut de Pharmacologie Moléculaire et Cellulaire CNRS, INSERM, Université de Nice-Sophia Antipolis Valbonne, France
[f] Inserm, U1141, Paris, France
[g] Univ. Paris Diderot, Sorbonne Paris Cité, UMRS 1141, Paris, France
[h] Pôle de Psychiatrie, Centre Hospitalier Régional Universitaire Carémeau, Nîmes, France
[i] Département des Urgences et Post-Urgences Psychiatriques, Centre Hospitalier Régional Universitaire, Hôpital Lapeyronie, Montpellier, France
[j] Laboratoire d'Immunologie, AP-HM, Hôpital de la Conception, Marseille, France

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ABSTRACT

Cytokines produced by both immune and non-immune cells are likely to play roles in the development and/or progression of psychiatric disorders. Indeed, many investigators have compared the blood cytokine levels in psychiatric patients with those of healthy controls or monitored their levels in patients during disease progression to identify biomarkers. Nevertheless, very few studies have confirmed that such cytokines remain stable in healthy individuals through periods of weeks and months. This is an important issue to consider before using blood cytokine levels as biomarkers of disease traits, disease state, or treatment response. Although multiplex assay technology represents an advance in identifying biomarkers because it allows simultaneous examination of large panels of analytes from a small volume of sample, it is necessary to verify whether these assays yield enough sensitivity and reproducibility when applied to the blood from neuropsychiatric patients. Therefore, we compared two multiplex immunoassays, the bead-based Luminex® (Bio-Rad) and the electro-chemiluminescence-based V-plex® (MesoScaleDiscovery), for the detection and quantification of 31 cytokines, chemokines and growth factors in both the sera and plasma of patients with major depressive episodes (MDE) and age- and sex-matched healthy control subjects during a 30-week period. Although both platforms exhibited low coefficients of variability (CV) between the duplicates in the calibration curves, the linearity was better in general for the V-PLEX® platform. However, neither platform was able to detect the absolute values for all of the tested analytes. Among the 16 analytes that were detected by both assays, the intra-assay reproducibility was in general better with the V-PLEX® platform. Although it is not a general rule that the results from sera and plasma will be correlated, consistent results were more frequent with the V-PLEX® platform. Furthermore, the V-PLEX® results were more consistent with the gold standard ELISA simplex assay for IL-6 in both sera and plasma. The intra-individual variability of the measurements, among the sera and plasma for the 4 samples harvested from each healthy individual, was low for Eotaxin, G-CSF, IL-4, IL-7, IL-9, IL-12p40, IL-12p70, IL-15, MIP-1β, PDGF-BB, TNF, TNF-β and VEGF, but intermediate or high for IFN-γ, IL-6, IL-8, IL-10, and IP10. Together, these data suggest that extreme caution is needed in translating the results of multiplex cytokine profiling into biomarker discovery in psychiatry.

1. Introduction

Cytokines belong to a large family of polypeptides that are produced by various cell types in the body, including the cells

* Corresponding author at: 51 Bd. Pierre Dramard, 13344, Marseille Cedex 15, France.
E-mail address: el-cherif.ibrahim@univ-amu.fr (E.C. Ibrahim).

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of the immune system. In particular, pro-inflammatory cytokines such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF) are rapidly secreted in response to a pathogen infection, whereas anti-inflammatory cytokines, such as IL-4, IL-10 and IL-13 have the opposite effects. Binding of the cytokines to specific receptors on the cell surfaces triggers events inside the cell that lead to altered gene expression profiles and cell function (Bezbradica and Medzhitov, 2009). In the last two decades, studies have shown that cytokines produced in peripheral tissues can access the central nervous system and interact with the cytokine network in the brain to influence virtually every aspect of brain function relevant to behavior, including neurotransmitter metabolism, neuroendocrine function, synaptic plasticity, and the neurocircuits that regulate mood, feeding, sleep, motor activity, motivation, anxiety and alarm (Capuron et al., 2001; Dantzer et al., 2011; Marshall and Born, 2002; Raison et al., 2006).

During the host immune response to pathogen invasion, the release of pro-inflammatory cytokines is usually transient and regulated by anti-inflammatory mechanisms. Consequently, the behavioral effects triggered by the increased serum cytokine levels have developped as an adaptive, temporary and controlled reaction of the brain to immune signals (Medzhitov, 2008). Nevertheless, when an immune challenge becomes chronic or unregulated, as occurs in patients who are receiving chronic cytokine treatments or those exposed to chronic medical illness or psychological stress, the cytokines may contribute to the development of clinically relevant behavioral symptoms and neuropsychiatric diseases, including major depression (Raison and Miller, 2013). In support of this hypothesis, depressive symptoms have been shown to be associated with increased serum and plasma concentrations of TNF, IL-6 and C-reactive protein (Dowlati et al., 2010; Goldsmith et al., 2016; Strawbridge et al., 2015; Valkanova et al., 2013). Most importantly, recent longitudinal studies have extended these cross-sectional observations by reporting that increased inflammatory markers in non-depressed individuals predict the later development of major depression (Gimeno et al., 2009; Pasco et al., 2010; van den Biggelaar et al., 2007).

Based on these observations, several authors have suggested that specific cytokines could be used as biomarkers in psychiatric diseases (Stuart and Baune, 2014), and even that cytokine-blocking reagents could be used to treat or prevent psychiatric symptoms (Fond et al., 2014; Kohler et al., 2014; Miller and Raison, 2015).

Recently, biomarkers for neuropsychiatric diseases have been classified into those of risk, diagnosis/trait, state, stage, treatment response and prognosis (Davis et al., 2015). In mood disorders, several studies have suggested that the chronic mild inflammation indicated by elevated C-reactive protein (Courtet et al., 2015; Rapaport et al., 2015; Uher et al., 2014) as well as the quantification of the levels of inflammatory cytokines such as IL-6, IL-10 and TNF (de Witte et al., 2014; Ducasse et al., 2015b; Haapakoski et al., 2015; Halaris et al., 2015; Rethorst et al., 2013) could prove to be useful biomarkers for improved diagnosis and the detection of treatment refractoriness (Strawbridge et al., 2015), as well as indicators of the risk of suicide. As an increasing number of inflammatory factors are tested, an emerging picture supports the idea that a complex network of cytokines is involved in the pathophysiology of disease such as major depression (Dahl et al., 2014).

It is known that many blood mediators present substantial individual variations among healthy subjects (Katsuura et al., 2011), and that acute psychological stress as well as acute exercise also produce robust effects on some circulating inflammatory factors in healthy people (Hallberg et al., 2010; Katsuura et al., 2010; Reihmane et al., 2012; Steptoe et al., 2007). In addition, most of the studies in this field have not considered the possibility that inflammatory factors measured in peripheral blood might exhibit a high degree of variability independent of any neuropsychiatric disorder. Some investigators have recognized the risk of making single measurements in healthy controls when assessing the variation due to antidepressant treatment in patients, but they emphasized that the previously obtained measurements were fairly stable, although this has not been documented in the literature (Halaris et al., 2015). Therefore, as a prerequisite for establishing biomarkers, investigators should define which factors actually exhibit stable levels in the circulating biofluids over days, weeks, and seasons in healthy adult subjects. Once this is established, it will be possible to distinguish real-time state-related protein concentration changes that are normalized by remission or by antidepressant treatment from the genetically and epigenetically driven physiological or stochastic variations that occur in healthy individuals. Moreover, even the previous results remained only partially reproducible. Although there are several possible explanations for these discrepancies (Zhou et al., 2010), reliability of the tools is one of the first steps required in biomarker development to avoid jumping to premature negative or positive conclusions (Niculescu et al., 2015).

Although the enzyme-linked immunosorbent assay (ELISA) technique has been widely used to measure serum cytokine levels in clinical studies, the recent development of multiplex immunoassays now offers the possibility of simultaneously measuring the levels of expression of large panels of proteins using a small volume of sample and at an affordable price (Altara et al., 2015; Butler et al., 2015; Guest et al., 2011; Halaris et al., 2015; Ho et al., 2015; Janelidze et al., 2013; Nielsen et al., 2015; Schwarz et al., 2012). Nevertheless, it is important for the new and constantly upgraded multiplex technologies to prove that their performance is at least equal to the 'gold standard' ELISA for each analyte tested in the combinations (Bastarache et al., 2014). In addition, the use of complex biological matrices such as sera or plasma must be used to mimic as closely as possible the biological test as it is planned to be used in clinical diagnostics. In biological human fluids, the seasonal, hormonal, and circadian physiological variability in the circulating concentrations of cytokines/chemokines/growth factors remains poorly investigated both in psychiatric patients as a function of their treatments as well as in healthy subjects. Furthermore, large differences exist among individuals (Agorastos et al., 2014; Biancotto et al., 2013; Mueller et al., 2012).

Among the various multiplex platforms, the fluorescent bead-based immunoassay Luminex® (Bio-Rad) and the electrochemiluminescence immunoassay V-plex® (Meso Scale Discovery) technologies dominate the market (Rosenberg-Hasson et al., 2014). Thus far, very few investigators have compared these technologies and evaluated the limits of quantification and intra-assay reproducibility for assay of human blood (Breen et al., 2011; Chowdhury et al., 2009; Fu et al., 2010; Nechansky et al., 2008; Toedter et al., 2008). Therefore, in the context of blood biomarker discovery in psychiatric disorders, we have aimed to evaluate whether the levels of circulating cytokines/chemokines/growth factors that have been identified as candidate biomarkers vary during periods of weeks and months in healthy individuals. For that purpose, we have tested the reliability of the two leading multiplex technologies, i.e. Bio-Plex 200 (Bio-Rad) with Luminex® Cytokine 27-plex assay and QuickPlex (V-PLEX®®) with V-PLEX® Proinflammatory and Cytokine panels (20 markers) using longitudinally collected sera and plasma from both healthy subjects and patients suffering from a major depressive episode (MDE).

2. Materials and methods

2.1. Populations

Blood sera and plasma samples were obtained during a 30-week prospective evaluation of a cohort of 10 patients with a