World Journal of Experimental Medicine

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DOI: 10.5493/wjem.v5.i3.188

World J Exp Med 2015 August 20; 5(3): 188-193
ISSN 2220-315X (online)

MINIREVIEWS

Multiplex planar microarrays for disease prognosis, diagnosis and theranosis

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Author contributions: Lea P solely contributed to this work.

Conflict-of-interest statement: None.

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Received: September 16, 2014
Peer-review started: September 16, 2014
First decision: October 28, 2014
Revised: June 5, 2015
Accepted: August 4, 2015
Article in press: August 7, 2015
Published online: August 20, 2015

Abstract

Advanced diagnostic methods and algorithms for immune disorders provide qualitative and quantitative multiplex measurement for pre-clinical prognostic and clinical diagnostic biomarkers specific for diseases. Choice of therapy is confirmed by modulating diagnostic efficacy of companion, theranotic drug concentrations. Assay methods identify, monitor and manage autoimmune diseases, or risk thereof, in subjects who have, or who are related to individuals with autoimmune disease. These same diagnostic protocols also integrate qualitative and quantitative assay test protocol designs for responder patient assessment, risk analysis and management of disease when integrating multiplex planar microarray diagnostic tests, patient theranostic companion diagnostic methods and test panels for simultaneous assessment and management of dysimmune and inflammatory disorders, autoimmunity, allergy and cancer. Proprietary assay methods are provided to identify, monitor and manage dysimmune conditions, or risk thereof, in subjects with pathological alterations in the immune system, or who are related to individuals with these conditions. The protocols can be used for confirmatory testing of subjects who exhibit symptoms of dysimmunity, as well as subjects who are apparently healthy and do not exhibit symptoms of altered immune function. The protocols also provide for methods of determining whether a subject has, is at risk for, or is a candidate for disease therapy, guided by companion diagnosis and immunosuppressive therapy, as well as therapeutic drug monitoring and theranostic testing of disease biomarkers in response to immunoabsorption therapy. The multiplex test panels provide the components that are integral for performing the methods to recognized clinical standards.

Key words: Simultaneous methods; Multiplex planar microarrays; Disease integrated panels; Theranosis; Prognosis; Diagnosis

Core tip: Multiplex planar microarrays integrated for simultaneous, quantitative methods of prognosis, diagnosis, and theranosis provide a powerful technology for comparative measurements of changes in disease states and risk analysis, especially for autoimmune diseases.

Lea P. Multiplex planar microarrays for disease prognosis,

diagnosis and theranosis. *World J Exp Med* 2015; 5(3): 188-193 Available from: URL: http://www.wjgnet.com/2220-315X/full/v5/i3/188.htm DOI: http://dx.doi.org/10.5493/wjem.v5.i3.188

INTRODUCTION

Recent investigation of genomic and proteomic technologies have provided data for pathological conditions of immune function. Studies of human body fluids have confirmed that antigens, their specific antibodies as well as inflammatory mediators, to be diagnostic markers specific for inflammatory, allergic and autoimmune diseases. These proteomic studies have diagnostic and therapeutic benefits[1]. Serum auto-antibodies and inflammatory indicators are detectable even before onset of clinical symptoms and also during the course of systemic and organ-specific dysimmunity. Immune serum-derived markers become predictive biomarkers of disease in healthy subjects and markers of disease activity and severity in patients. New multiplex diagnostic technologies being introduced in laboratory medicine[2] allow the simultaneous detection of several different auto-antibodies and biomarker analytes for screening purposes in high-risk groups. Auto-antibodies with demonstrated diagnostic and predictive roles in organ-specific systemic disease are reviewed[3]. Cohort studies have shown that patients may have carried auto-antibodies and other markers of dysimmunity for extended periods of time, not diagnosed until discovery of other related clinical symptoms, as reported by Bizarro[4]. Serum auto-antibody assays have clinical utility in autoimmune diseases, including Addison's disease, celiac disease, Crohn's disease, biliary cirrhosis, Hashimoto's thyroiditis and type-1 diabetes. Dysimmunity is genetically and immunologically complex. Early diagnostic biomarker discovery is becoming increasingly more important for early diagnosis and early treatment in these diseases. Proteomic analysis of body fluids provides a non-invasive methodology for early diagnosis in many different disease settings. Proteomics-based approaches have made steady progress into biomarker discovery and understanding of autoimmune, allergic and cancerous diseases[5]. Traditional diagnosis, diagnostic proteomic methods including targeted antibody-based protein arrays, which have been particularly informative in the field of autoimmunity, are compared by Wu and Mohan[6].

Auto-antibodies are specific markers of autoimmune disease and humoral autoimmunity. Fluctuations in acute phase reactants, complement, cytokines, chemokines and growth factors suggest systemic and cell-mediated dysimmunity. Clinical understanding of autoimmune and allergic diseases includes analysis of auto and allo-antibody class IgG, IgM, IgA and IgE immuno-globulins. Multiplex, simultaneous identification measures different auto-antibodies in sera of patients suffering from autoimmune diseases.

The simultaneous measure of correlating analytes in reduced test volumes of patient's samples and test reagents, plus faster diagnosis, lowers assay costs and provides more diagnostic results in comprehensive serological profiles. Improved quantitative data is obtained by high-throughput techniques such as planar multiplex microarrays for antibody and analyte diagnostic profiling[7]. Recently collected data demonstrate more accurate analytical sensitivity and reproducibility. Aspects of multiplex assay sensitivity, specificity, co-efficient of variation and data interpretation are becoming standards of these new and promising clinical applications[8].

Gibson *et al*[9] present results to demonstrate that discrete, individual, clinical, laboratory or radiological parameters are limited to accurately diagnose or predict disease outcomes. Biomarker protocol panels which diagnose at an earlier time point, indicate early diagnosis to guide therapeutic strategies for dysimmune conditions towards more effective clinical management. There is a growing need for deeper understanding of multi-factorial immune disorders. Proteomic platforms offering a multiplex approach are more likely to reflect the complexity of dysimmune disease processes. Diagnomic approaches aid in early detection and are beneficial in guiding immune disorder treatment towards earlier disease prevention. Integrated panels facilitate these aims by offering comprehensive etio-pathogenesis test panels to screen asymptomatic and diagnostic symptomatic subjects, for a multitude of autoimmune, dysimmune and allergic conditions early in their disease course to monitor disease activity and severity for more effective treatment.

ANALYTICAL ATTRIBUTES

Autoimmune diseases include twelve currently recognized systemic, sixteen systemic vasculitis, eight idiopathic inflammatory myopathies, four immune-mediated systemic, thirteen endocrine and reproductive, five hepatobilliary and pancreatic, four gastrointestinal, four cutaneous, six cardiovascular and pulmonary, eleven neurological, four occular, five renal and eleven hematologic autoimmune diseases to be active in serious illnesses found in almost every human organ system[10]. The increasing incidence of autoimmune pathologies, *e.g.* rheumatoid arthritis (RA) in aging populations, is reported in the scientific literature by Lea *et al*[2]. Allergies are increasing in prevalence, impacting the burden of health care costs. Allergic rhinitis, asthma, atopic eczema and food allergy are known as co-causes of chronic ill health[11].

Antibodies to endogenous self-antigens or foreign allergens continue to be detected and recognized as reliable disease biomarkers for autoimmune and IgE - mediated allergic diseases. For example[2], a patient may have RA without experiencing symptomatic pain, which

can delay early diagnosis. Typically, autoimmune diseases develop slowly. In RA. Early prophylactic detection leads to early treatment at efficacious drug concentrations, with potential for differential diagnostic pre-symptomatic recovery, especially in patients who respond to drug therapy and/or in whom immunosuppressive treatment would be of benefit. The treatment method of choice may include specific therapeutic antibodies which differ from standard autoimmune treatment. New and more specific biomarkers indicative of various dysimmune pathologies continue to be recognized and accepted as providing disease confirmation, facilitating differential diagnosis, disease activity monitoring and developing therapy guided by companion diagnostics. This key attribute relates to companion diagnostics, wherein molecular assays that measure levels of proteins, genes or specific mutations provide a specific therapy by stratifying disease status, selecting the proper medication and tailoring dosages to the patient's specific needs. As a result of more accurate proteomic assessment, the FDA (United States) continues to set more demanding guidelines. Therapeutic products require validation based on diagnostic test to conform to label, safety and effectiveness claims. The increasing need for companion or personalized tests is demanded by subsets of treated, positive responders to possible side effects.

Detection of single, discrete biomarkers in isolated test outcomes results in a number of patients as misdiagnosed for autoimmune disease. Incorrect test outcomes are directly caused by patient samples that may contain unstable biomarker antigens, auto-antibodies, endogenous antibodies, patient sample proteins and antibody-antibody reactions. These analytes are often unstable or interfered with in patient's test samples as a direct result of time dependent storage leading to breakdown and cross-contamination, including effects of proteolysis, oxidation and protein interaction. Repeat testing of such stored samples confounds result of confirmatory tests. New diagnostic methods and algorithms for immune disorders need to provide robust, simultaneous and comprehensive, qualitative and quantitative multiplex measurement for prognostic and diagnostic biomarkers specific for autoimmune diseases, while modulating companion diagnostic efficacy of theranostic drug concentrations. Assay methods help to identify, monitor and manage autoimmune disease or risk thereof, in subjects who have an autoimmune disease, or are cohort related. Therefore, it is a benefit to provide integrated indicator measurement using multiplex planar microarray diagnostic tests, including test panels designed for responder patient theranosis assessment, risk analysis and management of disease.

DIAGNOSIS MANAGEMENT AND THERAPY

The panels provide for diagnosis, management and therapy of immune disorders and dysimmunity. In particular, they provide diagnostic tests, test panel design, methods of use and kits for simultaneous integration of qualitative and quantitative multiplex planar microarray prognostic and diagnostic tests, therapeutic drug concentration tests, drug efficacy tests, companion diagnostics tests and methods of use applied in specific test panels for simultaneous assessment, determination of risk and management of immune disorders. Specific disease panels include tests for genetic predisposition and also measure biomarker responses to treatment efficacy, such as drug dose concentration and drug pharmaceutical efficacy. This data will determine the optimal drug dosage for each patient as the drug level is maintained at the pharmaceutical efficacy level for that patient. Test panels are also designed to facilitate earlier detection of multifactorial etiologies, differential diagnosis from related disease phenotypes and detect disease activity indices such as remission, flare-up, relapse and life-threatening organ involvement. In assessing the risk of whether a patient has or, at some point in the future is prone to develop an autoimmune disease, wherein the method includes: (1) obtaining a sample from the subject; (2) multiplex array measurement of inflammatory indicators, antibodies of various classes to a plurality of different endogenous antigens or allergens in the patient's sample; (3) comparing the same analytes simultaneously measured in reference subjects, or to previous blood draws from the same patient, and or comparing reference patients having a similar immune disorder; and (4) identifying the risk that the patient has, or might develop an dysimmunity based on the comparison in step (3) above. Risk is present when levels of one or more of inflammatory mediators or antibodies to the different antigens or allergens are elevated in the subject's sample as compared to samples from reference patients with clinically normal functions, and/or are elevated in, or at about the same level in the subject's sample.

The patients samples as contemplated in the above method, can be biological body fluids, including peripheral blood, serum, plasma, cerebrospinal fluid, synovial fluid, bone marrow, saliva and urine samples.

In another method to qualify immune disorder patients, as eligible to receive disease-modifying therapy, the method comprises: (1) obtaining a sample from the immune disorder patient; (2) measuring the levels of each of a plurality of different analytes in the patient's sample; (3) comparing the levels with the level of the same analyte measured in samples from reference subjects with clinically normal function, or from reference subjects having disease corresponding immune disorder; and (4) identifying the immune disorder patient as eligible to receive disease-modifying therapy based on the comparison in step (3).

In another embodiment, the analysis relates to a method for identifying and selecting a patient with immune disorder for therapeutic drug monitoring, wherein the method comprises: (1) confirming clinical decision

making and diagnostic algorithms so that multiplex planar diagnomic microarray tests and disease panels can be implemented to monitor treatment efficacy; (2) test panels are integrated for simultaneous, qualitative and/or quantitative prognosis, diagnosis and theranosis that may include measurement of drug concentrations and efficacy; (3) test panels allow patients and physicians to make scientifically based medical, individualized decisions about their disease and its therapy; (4) theranostic panels are defined as biomarker tests that specify the dose and efficacy of therapeutics in reference to a patient's genotype, disease phenotype and serological profile, in conjunction with bioinformatics technologies that analyze and interpret human disease and drug response interaction; (5) antibody and biomarker levels are compared as indicators of disease predisposition, stage of development, aggressiveness, drug efficacy, dosing and toxicity; and (6) test panels form a core component of a new personal medicine to transform clinical practice into definitive, diagnostic science.

Disease test panels incorporating biomarkers that confirm pre-symptomatic disease indicators allow targeted disease therapy. Disease stratification lies in correlation of molecular heterogeneity of disease and heterogeneity of response to therapy. The targeted drug must be present and act on maintaining or deteriorating disease symptoms of the patient state to show efficacy. This target would therefore identify a biomarker to determine whether the patient is a likely responder for treatment with a specific therapy. For example, a multiplex planar microarray fluorescent immunoassay, provides quantitative measurement for anti-TNF drug (Tumor Necrosis Factor) concentration for Infliximab, which is used to treat patients with Crohn's disease and RA[2]. Infliximab Research Use Only assays provide 100% specificity. Results of this testing correctly reported the absence of the drug in healthy serum samples, but accurately reported drug levels when expected to be present, for example in RA patients responding to treatment. This novel microarray assay system detects and measures TNFu blocker drug concentrations at high sensitivity and specificity. Measuring the level of the TNF-alpha for anti-TNF-α therapy also provides evidence of treatment efficacy. Additional markers for [rheumatoid (RA)] disease activity, when measured simultaneously with anti-TNF-alpha drug and TNF-units would indicate disease remission.

Biomarker protocol panels identify patients responding to specific therapeutics, as disease progression and treatment efficacy is monitored. Simultaneous measurement of several disease parameters improves benefits of treatment for a patient. These biomarker panels identify the most effective drug for a specific patient. Retrospective drug efficacy analysis following clinical trial with subgroup analysis, clarifies response variations.

Diagnostics are rapidly developing for the management of diseases and emergence of personalized medicine and drugs. As the uniqueness of each individual disease process is confirmed, so is a growing assurance that particular drugs work better in subsets of patients that could not previously he clearly identified or segregated.

Clinical trials have confirmed that subsets of patients may derive no benefit or were harmed by the drug being investigated. A primary benefit for improved, marker-driven patient selection is to determine who is most likely to benefit from a proposed treatment and to exclude patients likely to be harmed by a drug. Advanced protocol test panels efficiently combine development of drugs with companion diagnostic tests.

Accordingly, multiplex analyses are combined in test protocol formats to detect one or more indicators for refining diagnosis and patient selection for treatments. Panels of biomarkers need to be monitored during clinical trials to confirm effective therapy, while simultaneously testing for and confirming drug side effects.

Biomarker based test panels have utility in all phases of clinical work to better understand and confirm disease onset, progression, symptoms and mechanisms of drug action in a population prior to drug approval by regulatory bodies.

Validation for disease status prediction, or dysimmunity, results from clinical accuracy, reproducibility, predictive value and diagnostic efficiency in detecting disease. The challenge in disease validation is variation of biomarker response observed in patient populations. As newly validated biomarkers transition to clinical diagnosis, it should progress through analytical validation including clinical performance and specifications. Clinical validation includes recognition of early stage disease, disorders and clinical parameters to clearly differentiate from similar disease phenotypes. Assays are then tested systematically to standard, reproducible, high-throughput formats.

Autoantibodies to endogenous autoimmune antigens can be identified in apparently healthy individuals. As components of the idiotypic network, these immune-globulins may multiply to cause patho-physiological impact[12]. Such auto-antibodies against endogenous autoimmune antigens can be monitored using multiplex diagnostic tests, methods and kits. The quantitative measurements of these auto-antibodies and other inflammatory mediators clinically evaluate subjects at risk of developing or currently suffering from autoimmune disease or other immune disorders and also be used to monitor efficacy of a treatment regimen. Particularly advantageous are confirmatory testing of patients who exhibit immune disorder symptoms, screening tests for apparently healthy patients with no symptoms of dysimmunity, who are likely to have symptoms in due course.

DIAGNOSTIC TEST METHODS

Integrated diagnostic test panels provide for assessment, risk analysis and management of immune disorders. In one embodiment, the panels provide means for risk stratification when developing an immune

disorder and in assessing the response to treatment. The methods entail protocols for assaying samples from the subject for panels of analytes, wherein the presence of elevated levels of one or more of the different analytes indicates the presence or risks of an immune disorder. These methods incorporating multiplex planar microarray diagnomic panels, integrate for methods of prognosis, diagnosis and theranostic companion diagnostics. Physical examination, medical history and histopathology, completes a differential diagnosis for autoimmune disease. Tests can be carried out on asymptomatic patients or patients having risk factors or symptoms of the disease. When screening and a patient tests positive for elevated levels of one set of analytes, the patient is assessed for one or more additional confirmatory tests.

A method of assessing risk of vascular pathology includes assaying a sample for auto-antigen and for auto-antibody specific for that antigen. A presence of auto-antigen or auto-antigen reactive to-antibody, indicates risk of autoimmune disease. Increasing levels of auto-antigen and auto-antibodies to autoimmune disease antigens elevates risk for autoimmune disease. In one embodiment, the analytes being assayed together with auto-antibodies, may contain as part of a test panel, biomarkers that are not specific to a particular autoimmune disease, but indicate disease activity and a generalized dysimmune or inflammatory condition. Moreover, at some stages of inflammation and dysimmunity, the tested analyte level may decrease rather than increase over time, to provide valuable diagnostic or prognostic information for evaluating risk of autoimmune disease for endogenous disease specific analytes, for a specific immune disorder or dysimmune condition.

THERAPY SELECTION

Selected test panels determine whether a patient at risk or diagnosed with an immune disorder, is a candidate for immuno-suppressive or immuno-absorption therapy. The method assays a sample for a plurality of analytes, wherein the presence of altered levels of at least one of the analytes or biomarkers indicates that dysimmunity may be contributing to the subject's immune or inflammatory disorder and the risks thereof. Therapy can be applied together with one or more other tests, physical examination and taking of medical history in accordance with standard practice for differential diagnosis of immune or inflammatory disorders.

Approving a patient with immune disorder to receive therapeutic immune-modulation considers measured levels of serum autoantibodies or inflammatory mediators using a panel protocol of different endogenous analytes, in comparison to known reference samples with clinically normal function and confirmed reference autoimmune disease. A patient would receive immunotherapy if levels of at least one analyte are higher or lower than normal. A patient with an elevated level of at least one analyte may also be treated with immune-suppressive therapy or immune-absorption therapy in accordance with standard practice.

MULTIPLEX PLANAR MICROARRAY PANEL ASSAYS

Multiplex planar microarray disease assay panel markers, when printed into a microarray multiplex planar assay promote the integration of differential diagnosis, evaluating disease severity and duration, disease prognosis and monitoring response to treatment including therapeutic monoclonal antibodies, on an individual patient basis[13]. Each panel is comprised of biomarkers and therapeutic biologicals that are used in the diagnosis, differential diagnosis, prognosis and theranostic monitoring of a patient in a general disease classification, *e.g.,* autoimmune diseases. Some analytes are part of the clinical criteria for defining a particular autoimmune disease such as Rheumatoid Factor in RA. Additionally, several phenotypes of other autoimmune disease may be co-existant, confounding a diagnosis based on clinical grounds alone. For example, arthritic disease includes RA, idiopathic arthritis, polyarthritis, osteoarthritis and mixed phenotypes (psoriatic arthropathy, arthritis with inflammatory bowel disease). Each may have particular markers and associated treatments. Their inclusion into one panel aids in the differential diagnosis (Bayes Theorem; decision tree analysis) in terms of positive predictive values and odds ratios for ruling in or out a particular phenotype based on clinical presentation and results from laboratory testing.

Prognostic markers to ascertain the disease duration, severity, activity (remission or relapse), organ system involvement and response to effective treatment are also included in the multiplex planar microarray disease panel assays. Where applicable, measurable biological drugs, especially therapeutic monoclonal antibodies, also constitute theranostic data to monitor how diagnostic markers fluctuate in response to treatment and to measure pharmacokinetics of the biologics.

Diagnomic marker analyte classes include autoantibodies, serum immune-globulins, immune complexes, therapeutic antibodies detected as IgG, IgM or IgA immunoglobulin classes, as well as antigen capture formats for acute phase reactants, cytokines, chemokines, growth factors and inflammatory mediators. Panels for Autoimmune Disease include Arthritis, Vasculitis, Nephritis Hematologic Disorders, Celiac and IBD and Thyroiditis.

CONCLUSION

These multiplex planar microarray disease panel quantitative assays diagnose particular autoimmune disease, such as RA, vasculitis, nephritis, celiac disease and

Crohns' disease. Similar panels can be designed for other inflammatory conditions and allergic diseases. Multiplex Planar Microarray Assays can also be used for differential diagnosis of a particular disease subtype in a spectrum of related diseases as in RA *vs* osteoarthritis or juvenile idiopathic arthritis. Disease prognosis in specific disease or spectrum of related diseases, with detection of disease activity, severity and organ system involvement markers, is an additional benefit. Multiplex Planar Microarray Assays can be applied to pharmacokinetic studies and therapeutic drug monitoring of biological drugs used to treat cancer and autoimmune diseases (*e.g.,* therapeutic monoclonal antibodies), as well as biomarkers that fluctuate in response to treatment.

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P- Reviewer: Riccardi C, Song LT, Xavier-Elsas P
S- Editor: Tian YL **L- Editor**: A **E- Editor**: Jiao XK

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