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Genotype-dependent values of serum biomarkers in interstitial lung diseases

H.J.T. RUVEN[1] and J.C. GRUTTERS[2]

Interstitial lung diseases (ILDs) are a group of more than hundred heterogeneous disease entities sharing the pulmonary interstitium as primary focus of origin or manifestation of pathogenesis. Sarcoidosis (M. Besnier-Boeck), the most common ILD, is stereotyped for the presence of lung-localized, noncaseating granulomas of unknown origin (1). Stereotyped indeed, since sarcoidosis as a disease displays such a clear heterogeneity in partly overlapping characteristics or phenotypes that 'sarcoidoses' is a factually better description than sarcoidosis. For instance, Löfgren's syndrome, a spontaneously-resolving disease within a few months to two years and leaving no physical limitations has a clearly distinct disease course than chronic sarcoidosis leading to fibrosis of the lung and finally to the necessity of a lung transplantation. In the 'sarcoidoses', granulomas are also localized extrapulmonary making disease management even more challenging. Triggered by the clearly recognizable granuloma, consisting of a gathering of macrophages and T-cells, numerous speculations about the trigger or triggers causing sarcoidoses could not be substantiated. An environmental e.g. extracorporal trigger, obviously a chemical substance being most probably part of a micro-organism, in combination with the personal genetic layout and immune status, results in a patient's unique course of sarcoidosis (2). In many cases ILDs exhibit a unique disease course through the unique composition of an individual's genome and the involvement of not only the innate immune system, but also the dynamic processes of fibrogenesis and tissue remodelling in not only the environmentally exposed lungs but also in other organs.
Idiopathic pulmonary fibrosis (IPF), a devastating disease in the group of ILDs, is characterized by an unknown cause of onset of pulmonary fibrosis progressing towards inevitable lung transplantation. The severe manifestations of sarcoidoses and IPF, which are overlapping with both familial pulmonary fibrosis and sarcoidoses, require improvement of treatment possibilities (3, 4).

From a pulmonologist's perspective, established serum laboratory tests and new biomarkers need to be further explored to improve current daily clinical practice for patients with ILDs and to gain knowledge of the etiopathology of ILDs. From a clinical chemist's perspective, the sarcoidoses and IPF need to be explored to improve current daily clinical practice but also to gain insight in the knowledge of optimizing the application of serum biomarkers against a specific genotypic and phenotypic background of an individual or population. To meet editorial confinements, we will focus on the clinical chemist's perspective.

Genotype-dependent reference intervals and genetic variation between individuals

As a starting point, the relationship between genotype and serum concentrations of an analyte is not new. Gender-dependent differences between reference intervals of each laboratory test are, at least partly, differences caused by a shared, common genotype in each of the populations. Clearly, for each laboratory test, the absolute or relative contribution of genotype to the differences between reference intervals of two populations is essentially different. It is, however, a challenge to assess and really understand the contribution of genotype and the contribution of phenotype, of which the latter can be defined as the part remaining after removing the contribution of genotype.
Not new either is the relationship between genetic polymorphisms and its consequences for analyte levels in blood. Relatively new, however, is the potential to assess and to have access to genetic variation between large numbers of individuals. Genetic polymorphisms, including widely present single nucleotide polymorphisms (SNPs), can be related to analyte level, for which the selection of individuals and the definition of study populations are of major importance.

Clinical relevance of allele-specific reference intervals

Whether explicitly relating a genetic polymorphism to a reference interval indeed results in an improvement of clinical decision making has been demonstrated in a group of sarcoidosis patients in which the relationship between the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism and discordant classification of serum ACE activity has been studied (5). Splitting the reference interval for the widely used ACE laboratory test for assessing disease activity in patients suffering from sarcoidosis into three reference intervals based on ACE I/D allele, 8.5 percent of the measurements were classified in such a way that clinical interpretation of the serum ACE value changed. Though ACE activity as a measure of disease activity in sarcoidosis patients is still a matter of ongoing de-

Center for Interstitial Lung Diseases, Sint Antonius Ziekenhuis, Klinische Chemie[1] en Longziekten[2], Nieuwegein/Utrecht

E-mail: h.ruven@antoniusziekenhuis.nl

bate, it clearly demonstrates the significant improvement when changing from one interval to three allele-related intervals. Consequently, since previous studies on the clinical use of ACE activity in sarcoidosis have not taken ACE I/D corrected ACE values into acount, the discussion on ACE and sarcoidosis disease activity needs to be readily recuperated.
In our research on ILD we have not systematically focussed on identifying SNPs correlating to serum biomarkers. Nevertheless, using the candidate gene approach several cases have been identified as a 'by-catch'. Besides ACE and the ACE I/D polymorphism, several other pairs of serum biomarker and genetic polymorphism have been reported in ILD patient groups (6-8) and patients with breast cancer (9). Either SNPs contributing significantly to reference intervals of serum biomarkers are frequently present, or the candidate gene approach is a simple but effective strategy to identify SNPs which are clinically significant for defining and refining reference intervals.

Clinical relevance of allele-specific values of serum biomarkers

One step further in sharing the consequences of the results of allele-specific biomarker levels can be demonstrated by the serum biomarker Krebs von den Lungen (KL)-6 in sarcoidosis patients and healthy volunteers (6). Indeed reference intervals could be established for each of the three alleles of the 568 A/G polymorphism in the MUC1 gene resulting in 1,6 times higher KL6 levels in the group of 568 GG individuals compared with the group of 568 AA individuals. Interestingly, in sarcoidosis patients, in which KL6 is increased, supposedly as a result of disruption of the integrity of the alveolar-capillary membrane and regeneration of epithelial cells, the factor of 1,6 still holds between the sarcoidosis patients carrying 568 AA and 568 GG though absolute KL6 levels are 4 to 5 times higher.
When measuring YKL-40, a biomarker for inflammation, remodelling and fibrosis, in sarcoidosis patients and healthy controls, allele-specific (CHI3L1 -329 G/A) reference intervals in healthy controls clearly differ from each other whereas the differences in the slightly increased YKL-40 levels between the same allele groups in sarcoidosis patients seem to disappear (10, 8).
The results in sarcoidosis patients with KL-6 and YKL-40 and the relationship with a SNP in both coding genes demonstrate the possibility that the impact of an allele on analyte level in a healthy population may change under pathological conditions. It is tempting but, in brief, too bluntly to hypothesize that the change of impact of an allele under pathological conditions relates to the importance of a SNP in the pathology of the disease.

Z-scores

When clinical added value for correcting reference intervals of a laboratory test and SNP has been shown, the implementation of three allele-specific reference intervals for a laboratory test instead of one reference intervals can be carried out. In our view, the laboratory must meet the clinician's requirement for not being occupied by a triplication of reference intervals. To meet these requirements, the introduction of laboratory test reports with Z-scores related to each of the allele-specific reference intervals may be an efficient and effective measure. Essentially, the clinician is not interested in the allele composition of the SNP though it might very well be that this genetic information itself may prove to be a risk factor. Determination of CHI13L1 -329 G/A, the gene coding for YKL-40 can be used to report YKL-40 Z-scores based on -329 G/A-specific reference intervals. Less fortunately, -329 G/A neither could be related to predict survival in IPF nor was shown to be a disease susceptibility marker (8). So far, however, several other SNPs did not correlate with a serum biomarker but have shown to be related to disease activity and prognosis (7, 11-13).
Measuring SNPs and other genetic markers with the sole aim to improve the predictive values of frequently requested laboratory tests requires a multidisciplinary approach of laboratory specialists in clinical chemistry and genetics.

Concluding remarks

Refining a reference interval of a serum biomarker or a commonly used laboratory tests into three allele-specific reference intervals may lead to significant improvements for clinical decision making. It not only imposes the question which percentage of SNPs contribute - in a clinically significant amount - to reference intervals of laboratory tests, but also indicates the need to explore which polymorphisms have an high impact on reference intervals and whether it is worthwhile to incorporate allele-specific reference intervals in 'routine' laboratory testing.
Regarding genetics and biomarkers in ILDs, genotype-dependent values related to genotype-specific reference intervals enable us to investigate the pathogenesis. Either the loss or preservation of allele specificity of a serum biomarker during pathogenesis gives insight in the mechanisms underlying the disease. Given the number of SNPs and biomarkers, and the complexity of diseases, we would like to encourage multidisciplinary research of clinicians and clinical chemists on flawlessly-defined patient populations.

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Pharmacogenetics: from research to clinical implementation

R.H.N. van SCHAIK[1], L.L. ELENS[1], R.H.J. MATHIJSSEN[2], E.M.J.J. BERNS[2], B.Ch. STRICKER[3], L.E. VISSER[3], S.N. de WILDT[4], D.A. HESSELINK[5], T. van GELDER[5,6], J.N. van den ANKER[4], D. TIBBOEL[4], P.W. SCHENK[1,8] and J. LINDEMANS[1]

In today's medicine, patients are receiving drug therapy using dosages which have been established as the best dose, determined as an average in a large group of individuals. Although we take for granted that patients behave in a similar way on drug therapy, it is well known from clinical practice that interindividual variations exists in drug response. For drugs with a narrow therapeutic window, and/or drugs with potentially severe or even lethal side effects, this constitutes a major problem. Adverse Drug Reactions (ADRs) affect 2 million patients/year in the USA, resulting in 100,000 deaths annually. This makes ADRs the 5th most frequent cause of death. In fact, 7% of all hospitalizations are caused by ADRs (1-4). On the other side, of all drugs, only 25-60% are effective. A major part of the variability in drug response is thought to be the consequence of substantial interindividual variability in drug metabolism. This metabolism of drugs by the liver is partly determined by hereditary factors, with variant alleles of the same gene potentially encoding active, inactive or ultra-active enzyme activities. Using pharmacogenetics, being DNA analyses in genes encoding drug metabolizing enzymes and drug transporters, the challenge is to identify patients with these genetic variants, thereby predicting their corresponding metabolic capacity. With this information, genotype adjusted dosages, or another drug can be prescribed. This Personalized Medicine approach can improve healthcare by decreasing ADRs and improving effectivity of treatment, a topic of interest for patients and healthcare professionals with, in addition, substantial economical implications as well. The current challenge is therefore to characterize to what degree these genetic polymorphisms affect drug therapy. This would enable the identification of those pharmacogenetic markers that could help in routine clinical practice to explain, or preferably predict aberrant reactions to common drug therapies.

Departments of Clinical Chemistry[1], Medical Oncology[2], Epidemiology and Biostatistics[3], Intensive Care/Sophia Children's Hospital[4], Internal Medicine[5], Hospital Pharmacy[6], Erasmus University Medical Center, Rotterdam, The Netherlands

E-mail: r.vanschaik@erasmusmc.nl

Current affiliations: Division of Pediatric Clinical Pharmacology, Children's National Medical Center, Washington DC[7] and Dept. Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands[8].

Immunosuppressants: tacrolimus, cyclosporine and MMF

The most prevalent treatment in immune suppression for solid organ transplantation is a combination of mycophenolate mofetil (MMF), a calcineurin inhibitor (tacrolimus or cyclosporine) and glucocorticoids. Tacrolimus has a narrow therapeutic window. Underexposure may lead to acute graft rejection whereas overexposure can result in side effects, of which nephrotoxicity is the most troublesome. For