Cytomics in Predictive Medicine

a report by
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Professor Günter K Valet has been head of the Cell Biochemistry Group at the Max-Planck-Institut für Biochemie in Martinsried, Germany, since 1989. Prior to this, he headed the Mildred-Scheel-Laboratory for Cancer Cell Research from 1981 to 1989, at the same institution, as a joint venture between the Mildred-Scheel-Foundation and the Max-Planck-Society for the Advancement of Sciences. Professor Valet was elected president of the European Society for Analytical Cellular Pathology (ESACP) between 1994 and 1999 and of the Deutsche Gesellschaft für Zytometrie (DGfZ) between 1990 and 1994. He served as councillor for the International Society for Analytical Cytology (ISAC) between 1991 and 1995 and also between 1981 and 1985, has authored more than 170 scientific publications, was a member of several editorial boards of international scientific journals and has organised and co-organised international scientific congresses and numerous courses and workshops in flow cytometry. He was appointed Professor of Experimental Medicine at the Ludwig-Maximilian University of Munich in 1981, following habilitation in 1974 and post-doctoral work from 1972 to 1973 at the Scripps Clinic and Research Foundation in La Jolla, California. Professor Valet graduated from the medical faculty of the Ludwig-Maximilian University of Munich with an MD degree in 1968, after studying in Munich, Freiburg and Montpellier.

Patient-specific disease-course predictions with greater than 95% accuracy during therapy would be highly valuable for everyday medicine. If these predictors were available, disease aggravation or progression, frequently accompanied by irreversible tissue damage or therapeutic side effects, could potentially be avoided through early preventive therapy. The molecular analysis of heterogeneous cellular systems (cytomics) by cytometry, in conjunction with a pattern-oriented bioinformatic analysis of the multi-parametric, cytometric and other data, provides a promising approach to individualised or personalised medical treatment or disease management. As a consequence, better patient care and new forms of inductive scientific hypothesis development based on the interpretation of predictive data patterns are within reach.

Predictive medicine aims at the detection of changes in a patient's disease state prior to the manifestation of deterioration or improvement of the current status. Such instances may be related to multi-organ failure in sepsis or non-infectious, post-traumatic shock in intensive care[1,2] or the pre-therapeutic identification of high-risk patients in cancer cytostatic therapy;[3–6] but they are not limited to these instances alone. Accurate predictive measures would more effectively guide early anti-infectious or anti-shock therapy, as well as curative chemotherapy, in combination with stem-cell transplantation – potentially providing better survival chances for individual patients with concomitant cost-containment. Predictive medicine-guided early reduction or cessation of therapy may lower or abrogate potential therapeutic side effects. Other potentially important aspects of predictive medicine concern the recognition of pre-asthmatic and early rheumatic disease patients, as well as the pre-operative identification of patients with a tendency towards post-operative complications,[7] or coronary artery disease patients with an increased tendency towards restenosis or other complications.[8-10] Once this course of treatment is initiated, it becomes clear that the majority of everyday medicine is full of predictive issues. These

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are present in a variety of severe diseases, such as cancer, leukaemias, rheumatoid diseases, diabetes and asthma. However, they are also present with complex disease syndromes[11] and infections in newborns, paediatric patients,[12,13] adults or elderly patients, as well as bleeding risks during surgery or other affections. In many instances, individualised disease-course predictions for currently envisaged standard therapies would allow early, definitive, curative interaction by specific therapeutic measures, prior to the occurrence of irreversible tissue destructions with their inherent potential to incapacitate or compromise the patient in the long run.

Challenge

The obvious challenge is to define a generalised method of accessing or identifying individualised predictions.[14] Cell-oriented analysis is essential to this effort, because diseases are characterised by their significant deviations from the normal molecular processes in cellular systems or organs. The determination of the molecular phenotype of discrete cell populations by single-cell analyses is therefore particularly promising and represents evidence-based medicine at the cellular level. Molecular cell phenotypes emerge as the result of a patient's genotype and cellular environment, including exposure to disease-inducing agents. While the genotype seems the most important factor at first glance, the medical reality indicates that exposure to disease-inducing causes is frequently more important for disease generation than the genetic background. This is exemplified by individuals who are genetically highly susceptible to allergy or rheumatoid arthritis but unlikely to develop a disease without exposure to allergen, while heavy exposure may provoke the disease in relatively genetically resistant individuals.

Cytomics

As the cytometric analysis of the cellular heterogeneity in the expression of multiple molecules in the context of cytomes (in the cellular systems, organs and body), cytomics accesses a wealth of information on the molecular cell phenotype of specific diseases. The term cytomics carries a disciplinary aspect, as it was coined by molecular botanists[15] in analogy to genomics and proteomics, to describe the analyses of the genome, proteome and cytome (http://www.genomicglossaries.com/content/omes.asp). In contrast to the earlier term system cytometry, cytomics is method-independent. Members of a number of national and international organisations are involved in cytometric investigations in this area, including the European Working Group on Clinical Cell Analysis (http://www.ewgcca.org), the Clinical Cytometry Society (http//www.cytometry.org) and the International Society of Analytical Cytology (http://www.isac-net.org), which have recently established a focus on the basic research-oriented cell biology, molecular biology, bio- and nanotechnology aspects of cytomics.

Medical Bioinformatics

The determination of multi-parametric, individual-cell molecular parameters by cytometry, along with multiplex bead assays, cell population and single cell-based microarray technologies, generates large amounts of data. However, a major challenge remains in the efficient and effective extraction of the relevant predictive medicine parameters. Currently, this information is often extracted in a fragmentary way, by computer-assisted identification and characterisation of a few cell populations or gene clusters of interest. Alternatively, all of the available information can be screened exhaustively by means of multi-parametric

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clustering,[17,18] data mining or other procedures[19] for diagnostic or prognostic information-using, hypothesis-driven analysis strategies. These frequently require mathematical, statistical or other assumptions that may unintentionally bias the results. Assumption-free algorithmic evaluation concepts, such as data sieving[14] (http://www.biochem.mpg.de/valet/classif1.html) as a bottom-up approach, seem of particular interest for the detection of unknown molecular disease mechanisms that are inaccessible to a priori top-down-oriented hypotheses. Following the identification of the important predictive data patterns, a major task will still consist of the consensus-driven development of standardised predictive disease classifiers for clinical purposes.

Patient Groups

The ultimate goal of high statistical significance of the results of clinical applications conflicts, to a degree, with the search for individual patient-predictive parameters through the collection of large amounts of multi-parametric information. A two-phase strategy is therefore appropriate. During the initial pilot phase, the greatest number of potentially relevant individual cell measurements in the multi-parametric information data set is collected in prospective studies on clinically well-characterised patient groups at an acceptable minimum of statistical stringency, such as a significance level of $P>0.05$ or $P>0.10$. The majority of uninformative parameters can be eliminated at this stage by data sieving or multi-variate analysis.[7,8] In the second phase, the remaining informative parameters for disease-course prediction are analysed in statistically large patient groups.[3,4] This provides exact numbers for the reliability of individualised disease-course predictions and eliminates pseudo-informative parameters that, for random statistical reasons, have slipped into the group of informative parameters during the first phase. Informative parameters may likewise have been lost for random statistical reasons in the group of non-informative parameters during the initial phase. They may, however, be recoverable during the later deductive hypothesis- and concept-forming phase from the molecular context of the predictive parameter pattern that was determined during the first phase.

Prognosis and Prediction

The prognostic information contained in selected clinical and molecular parameters in large patient groups is typically utilised for risk and therapy stratification.[3] However, the well-acknowledged problem remains that, in general, therapy responders and non-responders cannot now escape being identified prior to therapy. The potential for pre-therapeutic identification of high-risk, non-responder patients by predictive medicine[4] is therefore of high clinical interest, because it helps to provide early therapeutic alternatives for high-risk patients by objective criteria.

Personalised Medicine

Unlike the prenatal screening for rare genetic diseases, which is restricted to a particularly important phase in human development, the cytomics-oriented approach to predictive medicine provides an entry into personalised or individualised medicine in a more general sense. Based on disease-course predictions at an accuracy level of greater than 95% or greater than 99%, current standard therapies can be modified by the clinician on objective grounds, according to the specific requirements of the individual patient. Clinical benefits will present themselves in terms of a better direction of diagnostic and therapeutic efforts to patients in need and a potential decrease in unwanted side effects because of an earlier cessation of therapy. Patients who do not respond to a specific therapy can be shifted to alternative therapeutic approaches immediately, thus lowering the risk of therapeutic failure and side effects and simultaneously reducing the costs of therapy.

Analytical Techniques

Predictive medicine is best implemented by cell-based measurements, e.g. by flow or image cytometry. Cell-oriented gene or protein arrays, as well as bead arrays for the capture of solute molecules from serum, plasma, urine or spinal fluid, are of equally high value.

The increasing miniaturisation of cytometers through semiconductor light sources and laboratory chip technology makes predictive medicine of interest for general, clinical and ambulant medicine as a point-of-care technology. In common with earlier driving forces in the continuously expanding cytometry field, predictive medicine represents a new challenge for clinicians, molecular biologists and cell-oriented bioinformatic scientists, in collaboration with the development of industrial instruments and assays. This article is intended as an initial effort towards encouraging research efforts in this new direction of cytometry. ■

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