9. POINT-OF-CARE MOLECULAR DIAGNOSIS: A NEAR FUTURE?

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9.1 Introduction

Is point-of-care molecular diagnosis a near future or not – it may not be too difficult to perceive and answer the question. The future of medicine is a logical consequence of the sequence of events, and some achievements and possibilities probably are currently closer than we can imagine. Some questions unavoidably emerge when considering the future of medicine and the role of molecular diagnosis in it, e.g.:

- What influences the development of medicine and medical biochemistry as tightly related professions (market economy?; science?; technology?)?
- What is the impact of market economy on the course of development of diagnostic methodology?
- What is the course of technological development? What are the predominant fields of development?
- What is the course of scientific development?

As many as 80% of medical decisions are made on the basis of laboratory findings. Therefore, it can well be stated that the results of *in vitro* laboratory tests are a vital aspect of medical decision making. However, the costs of *in vitro* laboratory diagnosis account for a negligible percentage of overall health care expenditures in the context of global market. Although still insignificant, this share has shown some increase in the last few years.

Investment in new reagents and technologies in general results in advanced methods used to detect various diseases and states. According to reports and estimates of renowned economy experts, in medicine these developments are currently most pronounced in the fields of infectious diseases, diabetes mellitus, cardiovascular diseases, and malignant diseases.

Considering technologies, in the last decade the most intensive development has been recorded in the methods of molecular diagnosis, e.g., various variants of the polymerase chain reaction (PCR) method, microarray and nanoarray technologies. This trend is anticipated to continue in the future, with ever increasing investments in this segment of market economy.

Hospital laboratories remain the most extensive consumer of diagnostic equipment and reagents. In the future, some non-institutional health care segments such as private practice and polyclinics will probably take a growing share. These segments will be subject to strict control of the tests and methodologies licensed to perform, and thus of the technologies available.

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9.2 The future of medicine

Technological development definitely plays the key role in the development of medicine. These exponential technological advancements make achievements once inconceivable to human mind now really accomplished. Novel technologies entail profound changes in medical profession and mode of approach to patient.

What trends are perceived in the development of modern medicine? Generally, the main idea is to make medicine a proactive rather than reactive in nature. What does it mean? According to Professor R.M. Satava from the University of Washington, USA, one of the pioneers in the field, the future of medicine will follow the 5P principle, according to which modern medicine is:

- predictive
- preventive
- point-of-care (POC)
- parametric
- personalized

Our understanding of factors that significantly influence the risk of particular disease development is expanded in parallel with the advent of the genetic screening methods and results acquired in large epidemiological studies. Predictive medicine tends to identify individuals or populations carrying some features in common and requiring some specific preventive measures, and diagnostic or therapeutic approach.

Preventive medicine integrates new concepts and is focused on the implementation of preventive measures in a population at risk, thus preventing the onset and development of the disease in the population. Such an approach is by far more efficient than the treatment of disease and is from the economic point perceived as a significant step forward in rationalization of overall health care costs.

There is no need to explain the POC aspect of modern medicine to medical biochemists. Modern medicine tends to the conditions that will enable continuous monitoring of all relevant parameters in a minimally invasive or completely noninvasive way, and in a setting most suitable for the patient, i.e. at home, at bedside, at work place, etc.

The current information technology enables observation and collection, analysis and understanding of the very complex systems of inter-related data. As such, it has created conditions for the development of so-called parametric medicine. Parametric medicine implies collection of many parameters, along with determination and monitoring of their changes over time, with due consideration of individual characteristics such as age, sex, race, etc.

Personalized medicine tends to adjust every diagnostic and therapeutic approach to the individual patient's needs. The constitution of each individual is absolutely unique, with a specific genetic code and phenotype, while living in inimitable environment. Thus, the hypothesis that every human being is distinguished by some aberration from all other individuals appears to be quite justified. Although the field has lately been the subject of in-depth research, it should by no means be perceived as a novelty in medicine. The concept of variability is very old indeed, dating from the times of Hippocrates, Galen and their disciples.

Sir William Osler (1849-1919), a Canadian physician, father of modern medicine, wrote: "It is more important what patient category is affected with a disease than what type of disease develops in a particular patient".

Accordingly, current medicine is based on care for patient. The medicine of tomorrow will make a step forward towards population screening and identification of individuals with a particular predisposition, their follow up over time, and implementation of preventive measures.

The development of molecular diagnosis is keeping up with the above mentioned changes in medicine. The molecular diagnosis of tomorrow will be (or already is?) a predictive, preventive, POC, parametric and personalized discipline.

9.3 Molecular diagnosis

Scientific advancements and knowledge of the sequence of genome, transcriptome, proteome and metabolome on the one hand, and technological development of novel sequencing platforms, microarray devices and other systems on the other hand make the basis of further improvements towards medicine of a new future era to come soon. What is the role of laboratory in such a new medicine? How will such a modern laboratory and new technologies meet the needs of emergency diagnosis?

Not more than some ten years ago, the idea of molecular diagnosis in emergency states seemed inconceivable and unfeasible. Today it is not so anymore. Since lately, POC systems for molecular diagnosis have been released to the market. These systems are intended for various fields, e.g., infectious diseases, pharmacogenetics and genetic testing.

One of these systems is the GeneXpert® released by Cepheid (Sunnyvale, CA, USA), proclaimed product of the year by the Small Times Magazine in 2005. It is the first system that allows for the process of isolation, purification and concentration, followed by DNA amplification and detection to be performed in a fully integrated and automated manner. While the current DNA technology involves several completely separate steps, and requires specific equipment and properly trained personnel for considerable manual manipulation over 1-3 days, the GeneXpert® real-time PCR system enables the desired sequence to identify within 30 minutes. These systems were initially employed for rapid analysis and detection of the potential biological war and terrorist agents at the national level within the United States Postal Service. Thousands of these systems were mounted across the United States, and the GeneXpert® system has since been certified and validated as an efficient anti-terrorist technology. GeneXpert® uses PCR technology for rapid analysis of air samples and for detection of DNA in traces originating from the bacterium *Bacillus anthracis* spores.

Cepheid Co. has also developed other methods for detection of various pathogens such as group B streptococci, some enteroviruses, Epstein-Barr virus, cytomegalovirus and methicillin-resistant *Staphylococcus aureus* (MRSA). In addition to this microbiological panel, some other genetic tests, e.g., rapid method for detection of BCR/ABL translocation, have recently been launched.

DNA isolation by the GeneXpert® system is based on the principle of ultrasonographic destruction of cell membrane or bacterial spore wall. The complete procedure proceeds in

disposable cartridges that contain mixing needles and physically separated chambers for different process phases. Upon membrane destruction, DNA is extracted into a microfluid column coated by DNA probes that bind the released DNA, while cellular debris passes and flows out through the column. The bound DNA is then released from the binding site and washed into the system segment for amplification. Various sample types and amounts (up to 5 mL of sample!) can be applied onto the device. Blood, tissue and various swabs can be used as a sample. Results can be obtained in no more than 30 minutes, whereas RT-PCR analysis takes about 2 hours.

Besides Cepheid, some other manufacturers also offer equipment for rapid molecular diagnosis testing (Table 9.1.). These instruments have not yet been routinely used; however, there are numerous ongoing clinical studies the results of which will make the basis to decide on the potential justifiability of introducing these tests in routine practice.

Manufacturer	Seat	URL address
Cepheid	Sunnyvale, CA	www.cepheid.com
Enigma Diagnostics	Witshire, England	www.enigmadiagnostics.com
LGC	Middlesex, England	www.lgc.co.uk
IQ^{uum}	Allston, MA	www.iquum.com
Nanosphere, Inc.	Northbrook, IL	www.nanosphere-inc.com
Lumora Ltd.	Cambridge, England	www.lumora.co.uk
Nanogen, Inc.	San Diego, CA	www.nanogen.com
HandyLab, Inc.	Ann Arbor, MI	www.handylab.com
IMM	Mainz, Germany	www.inn-mainz.de

Table 9.1. *List of manufacturers of point-of-care equipment in the field of molecular diagnosis (from Holland CA , Kiechle FL. Curr Opin Microbiol 2005;8:504-9.)*

9.3.1 Rapid detection of the cause of sepsis

Sepsis is a syndrome characterized by systemic response to infection which, if not appropriately treated, may lead to multiorgan dysfunction and death in a very short period of time. Sepsis is the third leading cause of death in the world. Eighteen million people are affected with sepsis *per* year, and every day 1400 patients die from sepsis worldwide.

The costs of treatment for severe sepsis account for almost 40% of overall intensive care unit (ICU) expenditures, amounting to 7.6 billion EUR in Europe and 16.7 billion USD in the USA. Thus, sepsis is one of the major challenges for ICU physicians. Therefore, the Surviving Sepsis Campaign was launched at the international level in 2002, with the objective to reach the fastest possible detection and diagnosis of sepsis at all hospital wards. As in sepsis the probability of survival correlates with the time of antibiotic therapy initiation, it is of utmost importance to introduce intravenous antibiotic therapy as early as possible, i.e. within the first few hours of the diagnosis of sepsis.

One of the major problems in the approach to sepsis patient is related to the choice of appropriate antibiotic, which should be based on proper identification of the causative agent in order to be efficient. According to literature data, more than 25% of patients receive inappropriate therapy. Microbiological methods are too time-consuming for this purpose. Traditional methods for detection of the causative agent take several days and have inadequate

diagnostic accuracy. Fungal infections are especially difficult to identify as the cause of sepsis (Figure 9.1.).

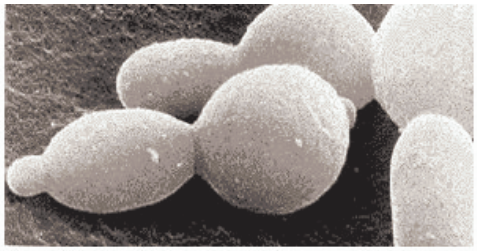

Figure 9.1. *Candida albicans as viewed by electron microscope (with permission from Dr. John Bennett).*

At the beginning of 2006, Roche released LightCycler SeptiFast, a new diagnostic kit for rapid detection of the cause of sepsis. It is a very rapid and reliable procedure to detect and identify as many as 25 most common causes of sepsis, which account for some 90% of sepsis cases. This method of pathogen type analysis is based on the principle of real-time PCR, as a method of amplification and melting curve analysis. The turn around time (TAT) from the initiation of sample processing to the result reporting is somewhat more than 5 hours. The first step of sample preparation takes 2 hours, followed by sample analysis that takes some more than another 2 hours. The last step is agent identification by use of computer program and finding report.

The example of the SeptiFast test points to the current achievements in the molecular diagnosis methodology. In formal terms, this method could not be included in emergency tests because it does not meet the professional criteria (TAT <1 h). Yet, the method definitely means a breakthrough as compared with the traditional microbiology methods, reducing TAT from several days to only a few hours. At present it appears irrelevant whether or not it is short enough to proclaim the method an emergency test. Sepsis is a condition that requires emergency intervention from the physician and as such imposes the need of the earliest possible diagnosis. If we assume that emergency means as early as possible, then in sepsis the earliest possible result is currently available in 5-6 hours, and from this point of view this test could be considered an emergency test.

It certainly is not the end of technological development. It can be anticipated that quite soon it will be possible to identify the cause of sepsis within a shorter period of time. The shortcoming of the current method lies in the procedure of sample preparation, which is too time consuming due to the still existing technological limitations. Some other manufacturers have already significantly reduced this step by use of the above mentioned methods of ultrasound destruction of cell membrane.

9.4 Pharmacogenetics

Among other fields where molecular diagnosis is employed for emergency diagnosis, mention should be made of pharmacogenetics. A rapid method to identify genotype which determines the patient's therapeutic response may occasionally be of crucial importance indeed. In this way, the incidence of unwanted and toxic drug effects that occur due to inappropriate therapeutic drug dosage and may pose vital threat for the patient would be significantly reduced. Target pharmacogenetic analysis prior to therapy introduction will certainly be routinely performed in the near future. These studies belong to the aspect of personalized

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medicine, described in the Introduction section. The main obstacles to these developments are not technology related. There are no technological limitations to halt or hamper the implementation of emergency pharmacogenetic analysis in clinical practice. The adoption and introduction of novelties in clinical routine need to be founded on strong arguments according to the principles of evidence based medicine. Large randomized controlled clinical trials have to be conducted on many of the known pharmacogenetic polymorphisms to demonstrate the justifiability and efficiency of this approach in clinically relevant situations.

9.5 Conclusion

The systems enabling the use of molecular diagnosis methods in emergency are yet to take hold in daily routine; however, it is just a matter of time for them to become a generally accepted standard. There also are some obstacles to overcome, which is not impossible but we have to be aware of them in order to approach and master them successfully. If molecular diagnosis is to be performed beyond central clinical laboratory and after hours, it will require proper training of the personnel working on these instruments. Also, the economic aspect of such changes needs to be taken in consideration, i.e. which systems from the ample offer to choose? Who will bear the cost of these tests? Are these tests cost-effective considering overall health care cost *per* patient? Medical biochemists should not only take the role of silent witnesses but also of active participants and visionaries of this technological and information revolution. We have to keep abreast with the fast progress and acquire due education and training in line with the high professional standards to be able to offer the latest and most sophisticated diagnostic methods to our patients when necessary.

Recommended literature:

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