Clinical Chemistry

Biomarkers of Brain Injury in Cerebral Infections

Article:
U. Rohlwink and A. Figaji.
Biomarkers of Brain Injury in Cerebral Infections
Clin Chem 2014;60:823-834.
http://www.clinchem.org/content/60/6/823.abstract

Guest:
Dr. Ursula Rohlwink is a neuroscientist at the Pediatric Neurosurgery Unit at the Red Cross War Memorial Children's Hospital in Cape Town, South Africa.

Bob Barrett: This is the podcast from *Clinical Chemistry*. I am Bob Barrett. Central nervous system infections, including diseases like meningitis and encephalitis, are important public heath concerns across the globe, as they occur commonly and/or associated with high rates of mortality and morbidity. Measurement of biomarkers provide objective indicators of normal function or pathology, and can present information that may assist diagnosis, assessment of health condition, and evaluation of treatment safety and efficacy.

They are regularly used in other conditions and their role in central nervous system infections is gaining increased attention. A review paper in the June 2014 issue of *Clinical Chemistry* describes our current understanding of the role of biomarkers in central nervous system infections.

Dr. Ursula Rohlwink is the lead author of that paper. She is a neuroscientist working in the Pediatric Neurosurgery Unit at the Red Cross War Memorial Children's Hospital in Cape Town, South Africa, and she joins us today in this podcast.

Dr. Rohlwink, why are biomarkers of relevance in disease, and in particular of cerebral infections?

Dr. Ursula Rohlwink: Well, cerebral infections actually are very difficult diseases to treat, and this is the case for a number of reasons. Firstly, most of these patients often present with very nonspecific symptoms, which makes the disease very difficult to diagnose.

Secondly, the clinical and laboratory tools which we currently have lack the sensitivity and specificity to swiftly diagnose the illness, and also to discern the causative pathogen, and this is particularly relevant when there are a host of pathogens that could actually cause infection.

These tools also lack the accuracy to assess how severe the disease is. So if we take clinical status of the patient as an example, the clinical status may be due to both irreversible factors like brain damage, and reversible factors like a poor electrolyte balance. And these reversible factors will respond

immediately to intervention and are therefore not necessarily reflective of the severity of the underlying disease.

When it comes to radiological imaging, we find that these images will often only demonstrate the injury once it has already occurred, once it's permanent, and many of these imagining modalities are not often available at the primary healthcare facilities where most of these patients will present initially, and are also limited in resource-impoverished settings.

So these factors combined result in the delay of diagnosis and therefore also the delay in the initiation of treatment, and as we know this may significantly increase the risk of poor outcomes in these patients.

It also makes it quite difficult to prognosticate and therefore to direct limited resources where they are in most need.

So one of the reasons for all of this uncertainty is the fact that the pathophysiology of many of the cerebral infections is actually still quite poorly understood. The characteristics about the host, and by host I mean the patient, as well as characteristics about the pathogen which are unclear, and when the two come together and interact to cause disease, this generates even more questions. And this is where biomarkers can come in.

So biomarkers are measureable indicators of the dynamic disease process. So they can be measurable indicators of the host response, measurable indicators of the pathogen, and of the interaction between the two.

So studying them may enhance our understanding of the pathophysiology, and they may therefore eliminate windows of opportunity where we may be able to intervene to arrest injury, and biomarkers can also act as objective indicators of illness, which are disease specific and sensitive enough to make definitive and swift diagnoses, and whose temporal profile may reflect disease resolution or deterioration and therefore allow prognostication.

In addition, if a biomarker is very clearly associated with injury severity, it can also serve as a surrogate marker for the effectivity of a new treatment intervention, where you would find that a successful treatment leads to the decrease in biomarkers and therefore the decrease in injury, and an unsuccessful new treatment would result in absolutely no change or even an increase in biomarker levels.

So these are some of the ways in which biomarkers can be of relevance in cerebral infections.

Bob Barrett: Can you please tell us about some of the biomarkers that are being investigated in these conditions?

Dr. Ursula Rohlwink: So biomarkers can be investigated at various levels of illness. If we start further downstream in the pathological process, we can look at some broad, unbiased systems approaches, like genomics and transcriptomics, which provide a very in-depth information about the host in terms of their genetic vulnerability, into individual variability and immune response, pathogen-directed changes in host gene expression, pathogen-specific host genetic or transcription profiles, and they can also provide quite a lot of genetic information about the pathogen in terms of the pathogen's genetic makeup and variability.

So one gets quite a broad idea of the host, the pathogen, and the interaction on a genetic level and one can indentify biomarkers from amongst differentially expressed genes or transcriptional RNAs.

Further upstream we get proteomics, which interrogates which proteins are differentially upregulated in the disease of interest, across very a broad scope of proteins. But this technology actually produces very large volumes of data that require quite substantial bioinformatics power. So lot of research to date has focused on selected proteins, and these have included markers of the immune response and information like cytokines and chemokines; they have included markers of cell death processes, like apoptosis and necrosis; markers of vascular injury, like selectins and integrins.

And then further upstream there has been a lot of research into biomarkers of actual brain tissue injury, and these include S100B and neuron-specific enolase, which are markers of astroglial and neuronal cell death, and which are actually quite extensively discussed in the article.

We also have metabolomics, which is another systems based approach, and it provides the opportunity to identify unique metabolic signatures for different pathogens and for different brain infections, but this technology is still in its fledgling state and there's still more development that's required there.

So essentially Bob, there are many options; many that are currently being investigated, and many that are still under development.

Bob Barrett: Well, these biomarkers sound like the Rosetta Stone of cerebral injury. Are there limitations to their use or the information they provide?

Dr. Ursula Rohlwink: They do offer a great potential, but there are indeed limitations to biomarkers and it's important to be aware of them. It's very challenging to actually find the perfect biomarker. The ideal biomarker should demonstrate high sensitivity and specificity for the brain. Its release should be associated with irreversible brain injury and reflect the temporal profile of that injury.

It should appear rapidly in the serum, because that is a much easier biological sample to access than cerebrospinal fluid, which requires an invasive procedure like a lumbar puncture or surgery. And the biomarker should demonstrate limited variability based on age and sex and should ideally be quite easily and speedily quantified by reliable assays.

So this is quite a tall order for a perfect biomarker. When it comes to a biomarker for the brain one needs to acknowledge that the brain is a highly complex and heterogeneous organ, with multiple cell types and different regions and therefore the cell specificity of the biomarker is actually very important.

In addition, infections of the brain vary both in form and severity. Sometimes infections can be chronic; other times they are acute, and this will be bear on the kind of biomarker and the timing of sampling, whether they would be earlier or later in the disease process.

Evidence suggests that cerebrospinal fluid is definitely a more representative sample than blood for quantifying biomarkers of cerebral infection.

The size and the amount of the biomarker that infiltrates the bloodstream is limited by the blood-brain barrier. And so the serum values are a function of brain injury, as well as the function of the degree of blood-brain barrier disruption. But even within the cerebrospinal fluid variability exists. Biomarker levels may be influenced by the distance between the affected area and the CSF compartment; they are affected by regional variability of biomarker proteins in the brain; and also by degradation of proteinases in the parenchyma or the CSF.

In addition, I think it's very important to remember that biomarker analysis is purely a quantitative measure and it cannot reflect both the qualitative and the quantitative functions of the brain. So these are issues which represent limitations to biomarkers of the brain specifically, but they are also issues related to patients in general, because there is considerable heterogeneity in patient characteristics and some studies have found differences in biomarker concentrations based on age, sex, and race.

In addition, not all patients actually have the same disease patterns or baseline immune characteristics, and as I mentioned earlier, there are many genetically driven differences across individuals.

On the methodological front, one also needs to be careful of collecting, storing, and processing samples in a way that's specific to your biomarker of interest, as these processes will actually differ from one biomarker to the next.

There is a variety of testing platforms that are currently available and different labs choose to use different technologies and as a result biomarker reference values and these concentrations are often not comparable across different platforms, and this makes a generalizability of biomarker concentrations quite difficult.

So it's important to consider these limitations when evaluating the usefulness of biomarkers and in planning studies, whether they be experimental or clinical, that will actually rely on biomarkers.

Bob Barrett: Well, how can physicians and laboratories overcome some of these limitations?

Dr. Ursula Rohlwink: Well, there are certainly a number of ways one can attempt to circumvent these limitations. On an international scale it would be ideal if large multi-center studies could be conducted through which standardized operating and testing procedures can actually be developed, and databases of reference and disease specific biomarker of concentrations could be established, and this would actually allow much easier comparison across studies or across clinical laboratories in different centers in the world.

But this is something that would require quite a well-orchestrated and resourced rich process. So what individual study groups and what individual laboratories and clinical centers can do is they can always ensure that they include a representative control group drawn from their population of interest, and they can use this control group to establish reference ranges against which they can actually compare their patients with disease.

Due to the heterogeneity intrinsic to the brain, intrinsic to the patient, and intrinsic to pathogens, using a panel of biomarkers is a very effective way of increasing their utility. And this panel can actually be designed according to the unique challenges of each disease.

I also think it's important to work towards combining the information provided by biomarkers with the information

that we already have available from current tools, like clinical examination, like physiological monitoring, and radiology, so that can we tackle disease with as many pieces of the puzzle in place if possible.

So if I can give an example, at our center here in Cape Town we are currently conducting a study on tuberculous meningitis, which is the most fatal form of TB, and which is an extremely difficult illness to treat and to manage. So the project combines markers of brain tissue injury with markers of inflammation and it evaluates their concentrations in the context of physiological data from brain oxygenation and intracranial pressure monitoring, as well as radiological data from the brain, from the spine, and the cerebrovasculature.

So in this way we are aiming to look at the full picture of TB meningitis in relation to outcomes in these patients. And this is an example of how clinicians, laboratories, and scientists can actually design studies that take biomarker limitations into consideration, but that use biomarkers to their greatest effect.

Bob Barrett: Well, finally doctor, let's look ahead: what would you consider an important future development of biomarkers in cerebral infection?

Dr. Ursula Rohlwink: There is a lot of tremendous work that is being done at the moment, but I really feel that we need to move towards getting biomarkers out of the laboratory and into hospital wards and clinics. The ideal is to have biomarkers that provide rapid results, that use testing methods that are user friendly and technically undemanding, that are inexpensive and sensitive, as well as specific to brain injury during that early phase of disease, because that is really the time where we can make the greatest difference in these patients. So that’s a very important future development which I see for biomarkers.

Bob Barrett: Dr. Ursula Rohlwink is a neuroscientist at the Pediatric Neurosurgery Unit at the Red Cross War Memorial Children's Hospital in Cape Town, South Africa. Her paper, “Biomarkers of Brain Injury in Cerebral Infections” appears in the June 2014 issue of *Clinical Chemistry*.

I am Bob Barrett. Thanks for listening.