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146

3rd Series

CEREBROSPINAL FLUID IN NEUROLOGIC DISORDERS

Edited by:
FLORIAN DEISENHAMMER,
CHARLOTTE E. TEUNISSEN,
AND HAYRETTIN TUMANI

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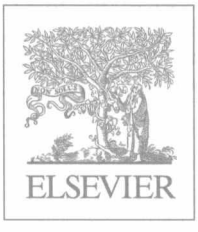

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British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

ISBN: 978-0-12-804279-3

Publisher: Nikki Levy
Editorial Project Manager: Kristi Anderson
Production Project Manager: Sujatha Thirugnana Sambandam
Cover Designer: Alan Studholme

Typeset by SPi Global, India

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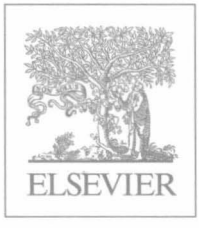

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Foreword

It could be argued that study of the fluid that surrounds the nervous system is as important as seeing it at autopsy or by modern imaging. It is remarkable that one had to wait until the 18th century for the "discovery" that the cerebrospinal fluid (CSF) is an actual fluid, thanks among others to Antonio Pacchioni in 1705 and Domenico Cotugno in 1764. Until then, it was predominantly thought that cerebral ventricles contained some vaguely aeriform *spiritus animalis* (spirit of the animal) and that mental functions were located within the cerebral ventricles. François Magendie introduced the term in 1842 and confirmed the presence of a connection between cerebral ventricles and subarachnoid space, as well as continuity of these spaces around the brain and spinal cord. Modern lumbar puncture was introduced by Heinrich Quincke and others at the end of the 19th century, and only then did the study of CSF start to have clinical applications. During the years since that time, there has been an enormous amount of research and clinical work on the CSF, but in-depth comprehensive publications dedicated to it remain relatively uncommon. It is therefore a pleasure to present this volume of the *Handbook*, which addresses all aspects of the CSF in neurologic disorders.

The volume opens with chapters describing in detail the anatomy and physiology of the CSF in animals and humans, as well the importance of CSF studies as biomarkers. It then focuses on the role of CSF studies in the diagnosis and clinical manifestations of many diseases, particularly multiple sclerosis, the dementias, strokes, malignancies, Guillain–Barré syndrome, and, of course, infections. Other conditions in which the role of CSF studies may be important include traumas, epilepsy, headaches, and psychiatric conditions. Changes in CSF pressure and hydrocephalus are discussed in detail. An account is also provided of current research involving the CSF that may allow fresh insights into these various neurologic disorders.

We have been fortunate to have as volume editors three distinguished scholars and clinicians – Florian Deisenhammer, Charlotte Teunissen, and Hayrettin Tumani. All three have been at the forefront of research on the CSF for many years. They have assembled a truly international group of authors with acknowledged expertise and produced this authoritative, comprehensive, and up-to-date volume. Its availability electronically on Elsevier's Science Direct site as well as in print format should ensure its ready accessibility and facilitate searches for specific information.

We are grateful to the three volume editors and to all the contributors for their efforts in creating such an invaluable resource. As series editors we read and commented each of the chapters with great interest and were impressed by the high quality of their work. We are therefore confident that both clinicians and researchers in many different disciplines will find much in this volume to appeal to them.

And last, but not least, we thank Elsevier, our publisher – and in particular Michael Parkinson in Scotland, and Nikki Levy and Kristi Anderson in San Diego – for their unfailing and expert assistance in the development and production of this volume.

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General aspects

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F. Deisenhammer, C.E. Teunissen, and H. Tumani, Editors
https://doi.org/10.1016/B978-0-12-804279-3.00001-0

Chapter 1

The use of cerebrospinal fluid in biomarker studies

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Abstract

Cerebrospinal fluid (CSF) is an extremely useful matrix for biomarker research for several purposes, such as diagnosis, prognosis, monitoring, and identification of prominent leads in pathways of neurologic diseases. Such biomarkers can be identified based on a priori hypotheses around prominent protein changes, but also by applying -omics technologies. Proteomics is widely used, but metabolomics and transcriptomics are rapidly revealing their potential for CSF studies. The basis of such studies is the availability of high-quality biobanks. Furthermore, profound knowledge and consequent optimization of all aspects in biomarker development are needed. Here we discuss current knowledge and recently developed protocols for successful biomarker studies, from collection of CSF by lumbar puncture, processing, and biobanking protocols, preanalytic confounding factors, and cost-efficient development and validation of assays for implementation into clinical practice or research.

INTRODUCTION

Cerebrospinal fluid (CSF) is the fluid immersing the brain. Due to its direct contact with the interstitial fluid, CSF reflects ongoing biology in the brain and therefore has important clinical and scientific use. Especially since brain biopsy is not possible, CSF is the best proxy to generate insight into ongoing pathologic processes. Consequently, CSF biomarkers are of great interest in research into neurologic diseases, not only to identify novel diagnostic biomarkers, treatment prediction biomarkers, or treatment response biomarkers, but also to generate insight into potential molecular players during early stages of disease evolution.

CSF has proven its use in clinical practice, exemplified by the use of oligoclonal bands for multiple sclerosis diagnosis (Teunissen et al., 2015), its crucial relevance in the diagnosis of infectious diseases of the central nervous system (CNS) and the recent strong advancement in use of amyloid and tau proteins for diagnosis of Alzheimer disease (AD) in clinical practice (Scheltens et al., 2016). These biomarkers, which are discussed in more detail in the other chapters in this handbook, have all contributed to an increased appreciation of the value of CSF to provide molecular endophenotypes. CSF -omics-driven studies have led to the identification of leads for pathologic mechanisms, as shown by metabolomics, proteomics, transcriptomics, and exosome research (Skog et al., 2008; Del Campo et al., 2014).

DEFINITION OF BIOMARKERS AND TYPE OF BIOMARKERS

In 1998 a Biomarkers Definitions Working Group from the National Institute of Health in the United States defined biomarkers as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (1998). In a more elaborate definition by Frank and Hargreaves (2003),

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three types of biomarkers are recognized: type 0 biomarkers are any signs or symptoms that correlate with known hallmarks of the natural course of a disease. Type 1 biomarkers have a relation with the response to a therapeutic intervention. Type 2 biomarkers (sometimes referred to as surrogate endpoints) represent markers that predict a certain clinical outcome and can thus be used in long-term studies as a substitute for a clinical outcome (Frank and Hargreaves, 2003).

There are several aspects that are important to consider when doing CSF biomarker research. CSF is distinct from other body fluids such as blood, among other reasons in that its collection is more invasive. Thus, possibilities for repetitive sampling are limited, affecting longitudinal research possibilities and monitoring. Studies have often been (and often still are) underpowered and, to overcome this problem, large collaborative networks have arisen to enable sufficiently powered studies, which have stimulated important progress in the standardization of many aspects of the research workflow, from collection procedures to analysis (Teunissen et al., 2014). Other unique aspects are its low protein concentration, and possible high stability. However, there is generally limited knowledge of stability of individual proteins in relation to preanalytic variation. Many of the stability data shown below have been obtained in recent years, in studies driven by the observed interlaboratory variation observed for the Alzheimer biomarkers.

A schematic representation of the stages in CSF biomarker studies is provided in Figure 1.1 and this chapter focusses on these stages. It starts with discovery/identification and verification, followed by validation, which usually includes alternative convenient assay development, analytic and clinical validation, and, when the studies focuses on biomarkers for clinical use, finally implementation. We will discuss methodologic aspects for each of these stages. The basis of all these studies are biobanks of optimally collected material. Therefore, after an introduction to the physiology of CSF, we will discuss standardized collection and biobanking protocols.

PHYSIOLOGY OF CSF

The major known function of CSF is to carry and cushion the brain to reduce pressure to the neurons residing in the lowest anatomic structures of the brain, protecting the brain against shocks or contact with the skull bone and against intracranial pressure differences due to changes in blood flow. Functions that are relevant for this review are transport of nutrients and hormones and waste disposal, in addition to a possible signaling function (Serot et al., 2012).

Sixty-six percent of CSF is produced by the choroid plexus. It further derives from the subarachnoid blood–CSF barrier structures, and from interstitial fluid drainage. Recent studies debate the classic view of circulating CSF (CSF production at the choroid plexus, directed bulk flow and absorption across the arachnoid villi) and suggest a more complex bidirectional flow of production and absorption of brain interstitial fluid and CSF across the walls of the blood capillaries of the CSF (Brinker et al., 2014). CSF is mostly an ultrafiltrate of blood, as 80% of the proteins in CSF originate from blood (Link and Tibbling, 1977; Reiber and Felgenhauer, 1987). CNS-specific proteins make up only 1% of CSF. However, not all blood constituents are present in CSF and since the CSF constituents can easily access brain cells, being separated by a thin ependymal layer around the ventricles, it is conceivable that this filtration is highly regulated. Moreover, peripheral changes (e.g., change in blood concentration due to dietary intake) may affect molecules that enter the CSF via ultrafiltration at the choroid plexus, while molecules from a CNS source will be less affected.

BIOMARKER SUBTYPES

Proteins

Over the last decades soluble protein biomarkers have been a major research topic for many different diseases that affect the CNS, including trauma, dementia, (non)infectious inflammatory diseases, and malignant processes (Brettschneider et al., 2009; Zetterberg et al., 2013;

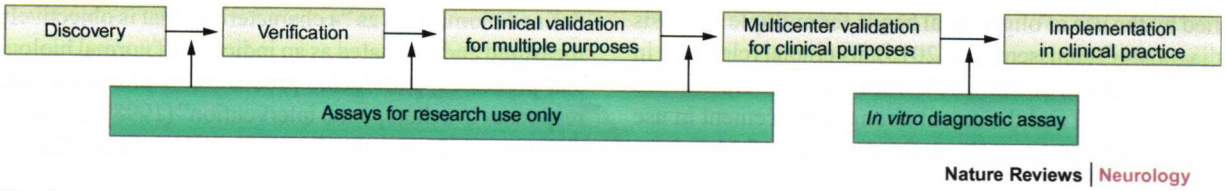

Fig. 1.1. Schematic representation of the process of biomarker development. The light-green boxes indicate the steps to be followed; dark-green boxes indicate the type (and quality) of assays that are typically used. The first three steps can be performed rapidly (in a couple of years), but the complete process to clinical implementation usually takes >20 years. (Reproduced from Teunissen CE, Malekzadeh A, Leurs C, et al. (2015) Body fluid biomarkers for multiple sclerosis – the long road to clinical application. Nature Reviews Neurology 11: 585–596.)

Raphael et al., 2015; Olsson et al., 2016; Shalaby et al., 2016). Protein biomarkers can be identified and validated by studying single or a small subset of proteins, or by high-throughput methods for proteomic analysis.

In some cases, presence of a protein biomarker is a direct consequence of a specific mutation that is absent in normal tissue but occurs in malignant processes. Point mutations in the isocitrate dehydrogenase 1/2 (IDH 1/2) enzymes are a well-known example in glioma. They are specific for malignant cells, as in the absence of disease the mutant protein would not be detected. Most protein biomarkers, however, are not unique in sequence or structure; instead either gene expression is altered, which results in changed protein levels compared to normal, or modifications such as phosphorylation alter the protein activity or function. A well-known example is tau protein, which is abundantly expressed in the neurons of the CNS. In AD, for example, the amino acid sequence of tau remains unchanged, but increased expression and phosphorylation levels are biomarkers for AD (Olsson et al., 2016). A protein biomarker may also represent several disease entities, which highlights the importance of thorough evaluation of clinical specificity. For example, glial fibrillary acidic protein is increased in CSF of patients with traumatic head injury, glioma, AD, intracranial hemorrhage, and multiple sclerosis (Szymaś et al., 1986; Jung et al., 2007; Husain et al., 2012; Petzold, 2015).

A common method to analyze protein biomarkers is the enzyme-linked immunosorbent assay (ELISA). This technique employs specific antibodies against a known target epitope; the presence of this target (i.e., protein biomarker) is then quantified with an enzymatic reaction. Nowadays, highly sensitive variants of the immunoassays exist, to enable analysis of low-level proteins in CSF.

Advances in the field of proteomics (mass spectrometry methods) facilitate the study of protein expression signatures in a certain body compartment such as CSF. Proteomics techniques can be used to identify many different (unknown) proteins in a sample. The protein concentration in CSF is approximately 200 times lower than in plasma, which poses a strong demand on the sensitivity of the detection methods but reduces background from blood-related proteins. Proteomic techniques are still being developed, with increased sensitivities and development of array-based methods (e.g., based on antibodies or aptamers).

Metabolites

Metabolites are the products and intermediates of cellular metabolism. Metabolites can have a multitude of functions, including energy conversion, signaling, epigenetic influence, and cofactor activity (Lu and Thompson, 2012; Wellen and Thompson, 2012). A well-known clinically used example of a metabolite biomarker is the measurement of glucose in CSF. This is performed in cases of clinical suspicion of infection, inflammation, or malignant process.

Metabolomics is the study of metabolite profiles. It utilizes mass spectrometry methods to analyze many different metabolites in a biologic sample (Markley et al., 2017). Nuclear magnetic resonance spectrometry is another useful tool in metabolomics (Markley et al., 2017).

Cell-free DNA

Normal cells release cell-free DNA (cfDNA) when they undergo apoptosis (Francis and Stein, 2015). cfDNA fragments that are found in peripheral blood are typically 150–200 basepairs (bp) long (Fleischhacker and Schmidt, 2007). Analysis of cfDNA as a biomarker in CSF thus far has its main application in the identification of somatic mutations in malignancies, or the detection of pathogens in infection. cfDNA fragments released from tumor cells are more variable in length than in normal cells due to different mechanisms in their derivation: apoptosis, necrosis, mitotic catastrophe, and autophagy (Jahr et al., 2001; Jin and El-Deiry, 2005). Somatic mutations that characterize a tumor can be detected in the isolated cfDNA (Bettegowda et al., 2014; Pan et al., 2015). Where there is infection with involvement of the CNS, cfDNA derived from pathogens can be used to identify the causal infectious agent (Grumaz et al., 2016; Weerakoon and McManus, 2016).

Polymerase chain reaction (PCR)-based methods can be used to analyze specific point mutations, but whole-genome sequencing methods can also be utilized to provide information not only about point mutations but also about copy number alterations and chromosomal anomalies (Leary et al., 2013).

Messenger RNA/micro RNA

Research into the role of microRNAs in homeostasis and disease is a relatively young field of study (Almeida et al., 2011). Several types of RNA have potential use as biomarkers of disease, such as messenger RNA (mRNA), small nuclear RNAs (snRNA), micro RNAs (miRNA), double-stranded DNA (dsRNA), and antisense DNA (Di Nallo et al., 2008; Fatima et al., 2015). The main focus for biomarker search is on mRNA and miRNAs. Both can be found in extracellular compartments, but mRNA is typically rapidly degraded in the extracellular environment while miRNAs are more stable (Touat et al., 2015). miRNAs are endogenous small

noncoding RNAs that function in mRNA regulation and mediate various cellular processes, such as differentiation and homeostasis (Lagos-Quintana et al., 2001). miRNAs are typically 22–24 nucleotides long, and fold on to themselves to form hairpin structures with structural and catalytic functions. They can be found encapsulated in extracellular vesicles (exosomes), as well as freely dissolved in bodily fluids such as serum and CSF, which suggests they may play a role in intercellular communication (Gilad et al., 2008; Weber et al., 2010; Baraniskin et al., 2012).

Aberrant miRNA expression patterns in CSF have been described for neurodegenerative diseases such as multiple sclerosis and AD, trauma, and CNS tumors (Baraniskin et al., 2012; Bergman et al., 2016; Chandran et al., 2017; Millan, 2017).

Specific miRNAs can be identified in the CSF through reverse transcriptase PCR (RT-PCR) or quantitative RT-PCR (qRT-PCR, real-time RT-PCR). Novel miRNAs can be identified with next-generation sequencing methods.

Exosomes

Extracellular vesicles are membrane-encapsulated packages that are secreted cells and can contain a plethora of biomolecules, including DNA, various types of RNA, lipids, proteins, and metabolites. These vesicles have an important function in intercellular communication and can readily be taken up by many cell types and modulate cellular behavior (de Vrij et al., 2015; Zappulli et al., 2016). Microvesicles are generally 1–2 μm in size and arise from shedding of the plasma membrane. Exosomes are smaller in size, with a diameter of only 30–100 nm (Harding et al., 1984; Raposo and Stoorvogel, 2013). They are generated intracellularly and originate from the inward budding of multivesicular bodies (Hanson and Cashikar, 2012; Andaloussi et al., 2013). The membrane surrounding the exosomes provides a unique protective environment that allows intercellular signaling with molecules that would otherwise not be secreted, such as insoluble proteins, proteins lacking a signal peptide, and intracellular or membrane-associated molecules (Bianco et al., 2009). Extracellular vesicles and exosomes are rapidly gaining interest as (containers of) biomarkers for many disease types, including neurodegenerative diseases and malignancies (Akers et al., 2015; Gui et al., 2015; Stuendl et al., 2016).

Exosomes can be isolated from bodily fluids with ultracentrifuge techniques, often combined with density gradients (Thery et al., 2006). Biomolecules of interest such as mRNA or DNA can then be extracted from the isolated exosomes and further examined.

Sample collection and biobanking: preanalytic variation

Preanalytic variation is an important factor that influences final biomarker results. This is important to control in large collaborative networks where differences in preanalytic handling inevitably occur, even when using the same protocols (Teunissen et al., 2010). Notably, the stability of a protein may be specific to the analytic technique in which it is measured, since certain epitopes may change under preanalytic conditions while others might not. For example, stability at different storage temperatures is often not well or not yet described for many individual CSF molecules. Such preanalytic studies may not by themselves lead to groundbreaking new scientific insights, yet they are the basis for studies that lead to such insights.

Nevertheless, stability may not be an issue for many CSF molecules; this may be due to the low protein content and low protease activity of CSF, as proven in studies directly assessing the stability of the CSF proteome (Berven et al., 2007; Jimenez et al., 2007). At the same time, the low protein content may be one of the defining factors of the relatively high absorption of some CSF molecules to lab plastics (Perret-Liaudet et al., 2012), which will be discussed in more detail below. Its low protein concentration further requires the application of highly sensitive analytic procedures, and existing assays that may be available for analysis of identified biomarkers in blood often need to be optimized for CSF analysis. Taken together, knowledge of CSF biology is relevant to interpret the relevance of CSF changes, and in addition CSF research poses strong requirements on knowledge and quality of preanalytic and analytic procedures.

Here we divide preanalytic aspects into two groups of relevant factors: patient-related, and preanalytic processing factors.

PATIENT-RELATED FACTORS

The first group of factors we describe are patient-related, such as age, sex, genetics, circadian rhythm, and dietary or lifestyle factors such as coffee consumption and smoking.

Age and sex

Age and sex are important defining factors for biomarker outcomes. Aging causes modifications of blood–brain barrier function and influences CSF turnover, thought to underlie neurodegenerative diseases (Zeevi et al., 2010; Chen et al., 2012). As such, age is often related to protein levels in the CSF, including the CSF biomarker concentrations of alpha-1-antitrypsin, transthyretin, gelsolin, neurofilament light, YKL-40, and TREM-2 (Chen et al., 2012;