Biomarkers are defined as "biochemical or molecular parameters associated with the presence and/or severity of a specific disease state" (1). Biomarkers are measurable by a variety of methods, including physical examination, laboratory assay, or imaging. In this narrative review, we focus on serum biomarkers for delirium, and discuss the advantages and limitations of this approach.

Three patterns of biomarkers emerge in relation to various disease states (1). First, a biomarker may serve as a risk marker for disease, that is, it is present before disease onset and identifies individuals at risk for disease. The apolipoprotein (APO) E4 allele represents a risk marker for early-onset Alzheimer's disease. Second, a biomarker may be a disease marker, that is, it rises with disease onset, and falls with recovery. CA-125 is an example of a disease marker for ovarian cancer. Disease markers are particularly important because they may provide clues as to the underlying pathophysiology of disease. Finally, a biomarker may be an end product of disease. Such a biomarker rises after onset of disease in proportion to the severity or consequences of disease indicating "damage" caused by the disease, such as creatine kinase in myocardial infarction.

Identifying accurate biomarkers for delirium may shed light on its pathophysiology. Biomarkers may also be helpful in delirium diagnosis, following its time course and severity, and determining its long-term sequelae. Comparing and contrasting biomarkers for delirium and dementia may lead to a better understanding of the interrelationship between these two syndromes, and enhance our ability to determine long-term cognitive effects that are attributable to delirium. Finally, biomarkers may provide the basis for pathophysiologically based treatment of delirium and for monitoring response to treatment. A summary of potential biomarkers for delirium appears in Table 1.

Serum Biomarkers for Delirium: Challenges

Studying serum biomarkers in delirium poses several challenges. First, delirium is a clinical syndrome; thus, there is no pathological "gold standard." Fortunately, in recent years, the definition of delirium has become well-established in the Diagnostic and Statistical Manual of Mental Disorders (26), and a number of diagnostic instruments have improved the accuracy and reproducibility of delirium diagnosis (27,28).

Second, consistent with its definition, delirium is a fluctuating process, creating difficulty in establishing its precise time of onset or termination. Thus, the timing of biomarker testing with delirium can be challenging.

Third, delirium is a central nervous system (CNS) process. The blood–brain barrier is felt to effectively segregate the CNS from the bloodstream. Therefore, the contention that serum biomarkers will be indicative of a CNS process can be challenged. However, delirium may be a CNS manifestation of drugs and other processes (e.g., infections, metabolic derangements) that cross the blood–brain barrier (29). In fact,
one conceptualization of delirium is a state of blood–brain barrier compromise (30). In either case, there is a theoretical rationale for considering serum biomarkers for delirium. Finally, and perhaps most challenging of all, delirium occurs primarily in acutely ill hospitalized older adults. Many factors affect hospitalization, including the nature of the acute illness, its severity, comorbidities, and medications. Fortunately, excellent predictive models for delirium that incorporate these factors have been developed for both medical and surgical patients, and can facilitate isolating markers for delirium rather than the underlying illnesses in which it develops.

**RISK MARKERS FOR DELIRIUM**

**Serum Chemistries**

In several validated risk indices, serum chemistries have been identified as risk markers for delirium. Inouye and colleagues defined a blood urea nitrogen (BUN)/creatinine ratio > 18 to be an independent predisposing risk factor for delirium in general medical patients (2). Elevated BUN/creatinine may be indicative of dehydration, congestive heart failure, poor oral intake, or other factors that predispose the patient to delirium. Marcantonio and colleagues defined preoperative “markedly abnormal serum chemistries” (sodium < 130 or > 150 mEq/L, potassium < 3.0 or > 6.0 mEq/L, and glucose < 60 or > 300 mg/dL) to be an independent risk factor for postoperative delirium (3). Others have defined perturbations of serum electrolytes, glucose, and renal function as both risk markers and causes of delirium (4). In addition to identifying persons at increased risk for delirium, these biomarkers may also assist in treatment or prevention.

**Genetic Markers**

Genetic material is available in leukocytes from centrifuged peripheral blood. Because of the increasing evidence of the interrelationship between delirium and dementia (31), one might predict that genetic risk markers for dementia might also be risk markers for delirium. Despite this, few studies have examined the relationship between the Apo E4 allele and delirium. No studies have been performed in medical patients, and in surgical patients, studies have focused on postoperative cognitive decline (5,6,32), a condition related to, but not synonymous with, delirium. These results are equivocal, with the largest study showing no relationship (32). If an association between Apo E4 and delirium is demonstrated, future studies should carefully measure and adjust (or stratify) for pre-illness dementia status to determine if this association is mediated by dementia, or whether Apo E4 confers risk for delirium independent of dementia.

Other than Apo E4, there are few data linking specific genetic alleles with delirium. The A9 allele of the dopamine transporter gene has been associated with delirium tremens in alcohol withdrawal (7), but this allele has not been examined in medical or surgical patients with delirium. Given the interrelationships between acetylcholine and dopamine in the CNS (31), and the success of antidopaminergic agents for treatment of agitation in delirium (31), this allele deserves further investigation.

**DISEASE MARKERS FOR DELIRIUM**

**Pathophysiology of Delirium**

There has been little research into the basic mechanisms of delirium. A major challenge has been the heterogeneity of the delirium syndrome and the populations in which it is studied. Early data suggest that different underlying mechanisms may pertain in different clinical situations (29). Although delirium may result from a neuroanatomic abnormality (e.g., stroke), the vast majority of cases are caused by an imbalance of key central neurotransmitters (Figure 1).
The most prominently implicated neurotransmitters in delirium have serum biomarkers, reviewed below.

**Acetylcholine and Serum Anticholinergic Activity**
A widely postulated mechanism for delirium is cholinergic failure (33). The first evidence for this mechanism came from case reports linking delirium to acute poisoning with anticholinergic drugs and demonstrating reversal of delirium with procholinergic drugs (33). Epidemiologic studies have found that patients with higher “anticholinergic burden” of their drug regimens are at greater risk for delirium (34). Estimating “anticholinergic burden” from a list of medications is imperfect because of interindividual variation in drug pharmacokinetics. Moreover, at least one small study suggests that some individuals may have anticholinergic activity in the absence of drugs (35). A bioassay has been developed that measures total serum anticholinergic activity (SAA) (36). Originally developed to measure the anticholinergic burden of drugs in psychiatric patients, SAA has been applied to delirium (36). It is a competitive binding assay in which serum competes with a standard muscarinic ligand for binding to whole rat brain homogenates. One study has demonstrated a close correlation between serum and cerebrospinal fluid anticholinergic activity, suggesting that these substances cross the blood–brain barrier (37).

Several studies have demonstrated a strong association between SAA levels and delirium in medical and surgical patients (8–11). Although most have been cross-sectional, one study found that decline in SAA was associated with delirium resolution (11). Further studies are needed to measure serial SAA levels and to correlate levels with the incidence, persistence, and resolution of delirium.

An exciting innovation is the development of muscarinic subtype–specific assays (38). Rather than relying on whole brain homogenates, these assays use recombinant human muscarinic receptors specific for the five subtypes, with subtype one postulated to be cognition related. Finding that SAA related to delirium is specific for cognition-related muscarinic subtypes will lend credibility to the use of SAA as a biomarker for delirium.

**Serotonin, Serum Amino Acids, and Melatonin**
A second potential mechanism for delirium relates to imbalance in the serotonergic system. Elevated CNS serotonin activity can be seen in hepatic encephalopathy and “serotonin syndrome” caused by acute withdrawal of selective serotonin reuptake inhibitors (29). More common in medical and surgical patients is central serotonin deficiency. CNS serotonin levels are dependent on tryptophan, which competes with large neutral amino acids, most notably phenylalanine, for transport across the blood-brain barrier. Accordingly, high serum levels of phenylalanine (common in postoperative or posttraumatic catabolic states), low serum tryptophan levels, and low tryptophan-to-phenylalanine ratios have been associated with delirium (12,13). Studies in this area tend to be small and cross-sectional (12); however,
one longitudinal study reassessed serum amino acid levels after resolution of delirium, but yielded equivocal results (13).

Serum amino acids are a challenging biomarker for delirium. They fluctuate widely depending on the protein content of the diet (29). They are expensive, and require specialized processing of the serum to ensure stability. Nonetheless, amino acids represent a potentially modifiable mechanism for delirium, and warrant further investigation.

A related biomarker that impacts the serotonergic system is melatonin, a hormone secreted by the pineal gland that is felt to be important in sleep–wake regulation. Melatonin could provide the link between delirium and disruption of the sleep–wake cycle (14). The evidence linking melatonin with delirium is limited. One small study examined urine (not serum) metabolites of melatonin and found normal levels in patients without delirium, high levels in those with hypoactive delirium, and low levels in those with hyperactive delirium (15). As melatonin can be administered, it warrants further investigation as a biomarker of delirium.

Inflammation

Inflammation has been associated with many morbid conditions in the aged population, including cardiovascular events (39), frailty (40), and Alzheimer’s disease (41). Moreover, delirium is common in systemic inflammatory states, including infections, cancer, and the postoperative setting. Inflammation leads to a breakdown of the blood–brain barrier (30), and has also been shown to decrease cholinergic transmission (42). Therefore, inflammatory markers (or cytokines) may be important biomarkers for delirium.

Several families of inflammatory markers exist, including the interleukins, interferons, and growth factors. Each plays a role in the inflammatory cascade, with some initiating the early response, others modulating it, and still others mediating the later recovery processes (43). Inflammatory markers are relatively easy to measure, and the advent of new multichannel analyzers allows simultaneous assessment of up to 30 biomarkers in 100 microliters of serum.

Existing studies of delirium and inflammatory markers are small, and are either cross-sectional or involve only two time points. One study reported a 30% delirium rate in patients receiving high dose interleukin-2 for malignancy (16). Studies performed in other medical and surgical populations have reported one or more inflammatory markers elevated more in delirious than in nondelirious patients (17–19).

Several caveats are worthy of mention. First, given the large number of inflammatory markers, it is important to consider issues of multiple statistical comparisons. Second, although C-reactive protein is viewed as a summary measure of inflammatory activity, it actually captures only one specific aspect of inflammation, which may not be the most relevant for delirium. Third, inflammatory markers tend to cluster into groups that rise and fall in response to stress with a specific time course, and the results must be interpreted accordingly. Finally, inflammatory markers may be confounded by other risk factors for delirium, such as age, type of insult, and comorbidity; thus it is important to control for these factors. The challenge is to isolate patterns of cytokines that capture the specific inflammatory response most related to delirium.

Chronic Stress, Catecholamines, and Cortisol

Chronic stress causes activation of the sympathetic nervous system, which in turn activates the hypothalamic pituitary axis and causes elevation in serum cortisol. Elevated serum and urinary catecholamines have been measured in alcohol withdrawal delirium, but not in more typical medical and/or surgical delirium (29). The link between delirium and cortisol has more evidence. Elevated cortisol levels have been associated with delirium in Cushing’s disease and high dose steroid treatment (29). Only a few small studies have examined the association between serum cortisol levels and delirium in general medical and surgical settings, and the results have been equivocal (20,21). When an insulin can be anticipated (e.g., surgery), another approach is to administer a dexamethasone suppression test. One report suggests that individuals who fail to suppress appear to be at increased risk for delirium (22). Individuals who fail to mount a cortisol response to stress may also be at risk. As with the other biomarkers, careful control of patient characteristics and the delirium insult will be required to confirm the importance of hyper- or hypocortisolism as a biomarker for delirium.

Gene Expression

All of the biomarkers described above are based on specific hypotheses about the pathophysiology of delirium. Another approach is to make no assumptions about pathophysiology, but rather to perform nonspecific gene expression analysis. Although this approach is exploratory, it has gained significant acceptance in the molecular biology community, particularly when beginning investigation in new fields. Because of the ease of obtaining it, the lymphoid fraction of peripheral blood is used most frequently for gene expression studies, although some might argue that more definitive results would be obtained from brain tissue.

Gene microarray analysis is a technique by which the expression of thousands of genes can be measured simultaneously (44). By comparing microarrays between persons with and without delirium, one can examine whether certain genes are “turned on or off” differentially during an episode of delirium. As with other biomarkers, it is important to control for patient and insult characteristics, which themselves may have effects on gene expression. Similar techniques are available to measure levels of serum proteins using large-scale proteomic scans (45). If a particular gene or protein appears overexpressed or underexpressed consistently in patients with delirium, additional techniques allow the investigator to identify the gene or protein of interest. This work is in early stages, and no biomarkers for delirium have yet been identified using these techniques.

End Products of Delirium

Although the sequelae of delirium remain uncertain, it is likely that at least some cases of delirium result in direct neuronal injury. Thus, serum biomarkers that detect neuronal injury or death may be appropriate for delirium
onset, delirium persistence, or adverse cognitive sequelae following delirium. Three such markers have been examined: neuron-specific enolase, S-100 beta, and neuronal tau protein. All are proteins that can be assayed from serum using standard enzyme-linked immunosorbent assay methods. They have primarily been studied in other forms of direct neural injury, such as stroke and head trauma, where they appear to reflect the degree of damage sustained (46–48). Only a few studies have been performed in delirium, with inconsistent results (23, 24). Neuronal injury markers, especially S-100 beta, appear to be predictive in postoperative cognitive decline (25). These neuronal injury markers may be particularly helpful for identifying individuals at higher risk for dementia after an episode of delirium.

**Biomarkers for Delirium: An Assessment of the Literature and Recommendations for Future Research**

The current literature examining serum biomarkers for delirium has generated several important hypotheses that require further evaluation with more definitive study designs. Among the weaknesses of the current studies include small sample size, cross-sectional design, inadequate characterization of baseline characteristics of the participants, and heterogeneous nature of the insult leading to delirium. Each of these weaknesses makes it difficult to isolate the impact of delirium on biomarkers versus other patient-related or illness-related factors.

A major advance in this field would be a large observational study in which delirium and biomarkers are measured before, during, and after the episode of delirium to correlate biomarker levels with both the onset and resolution of delirium. Careful selection of the study population, matching for baseline characteristics that might be associated with biomarker release, and controlling for the insult leading to delirium will improve interpretation of these data. These important steps can be facilitated by incorporating existing multifactorial prediction models for delirium in patient selection and analysis. Each of the classes of biomarkers described in this review are worthy of investigation, but those focusing on genetic risk, the cholinergic system, and inflammation may be most important for elucidating potential linkages between delirium and dementia. Such studies will increase our understanding of the pathophysiology of delirium, and ultimately improve our methods of monitoring and treating delirious patients.

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