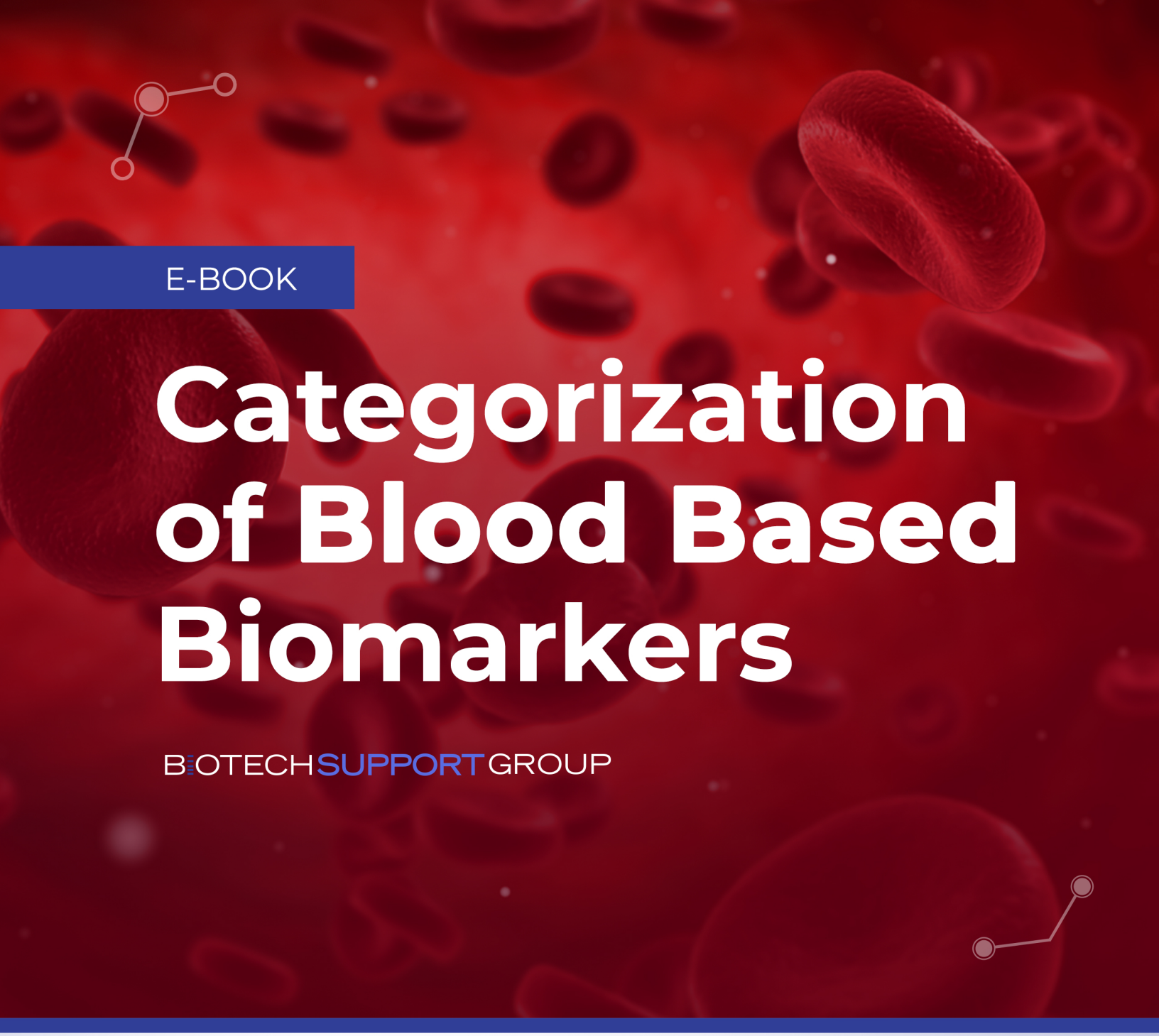


E-BOOK

Categorization of Blood Based Biomarkers

BIOTECHSUPPORTGROUP

Unleashing the power of proteomics to better understand the innate immune response to infectious and non-infectious inflammatory stimuli

BSG

Categorization of Blood Based Biomarkers -

Unleashing the Power of Proteomics To Better Understand the Innate Immune Response to Infectious and Non-infectious Inflammatory Stimuli

August 10, 2020

Chapters:

1. Healthcare Needs Biomarkers That Are Actionable
2. Blood is the best vehicle for biomarker information
3. Proteins are the ground truth of biology, but proteomic productivity can be improved
4. Proteome Categorization distills better information into useful biomarkers
5. Confluence of technologies, methods and strategies will improve proteomic productivity and generate actionable biomarkers for precision medicine
6. Efficient Sample Prep Enrichment Brings Better information for Blood

Learn how:

BSG's products and methods can help proteomic investigators explore all blood compartments.

BSG's fast and efficient sample prep products and methods allow quantitative monitoring of multiple blood proteins in the mid-high abundance range.

BSG's products enrich potential protein biomarkers of low-abundance to mid-abundance so as to improve linearity between peptide ion signals and protein abundances.

New categorization strategies for selection of innate immunity biomarker proteins in the mid-high abundance range can change characteristically with the onset of disease.

Past proteomic analysis based on antigen recognition can lead to egregiously misleading information on the status of protease regulation in the general blood circulation.

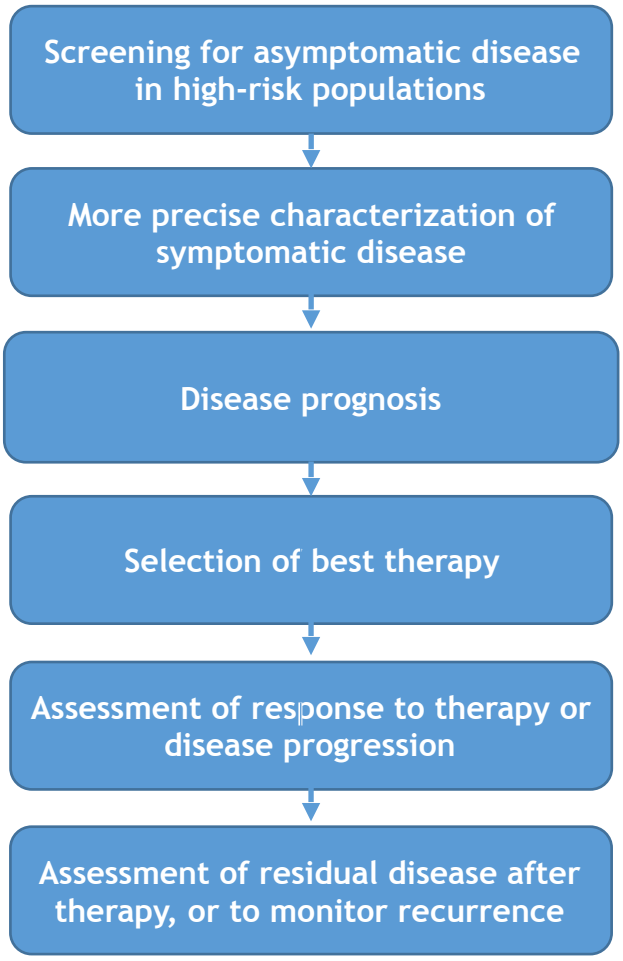
Unresolved inflammatory stimuli can lead to chronic disease and pre-dispose individuals to a severe acute response to new infectious exposure.

The convergence of proteomic technologies has made the task immediately available to understand the innate immunity proteome in the pathogenesis of pandemic infections, cancer, and autoimmune disease.

Unbiased data-driven proteomic analysis can quickly lead to development of the next generation of molecular tests for more precise and personalized treatment of patients.

Healthcare Needs Biomarkers That Are Actionable

Biomarkers are Desirable in All Stages of Health Management



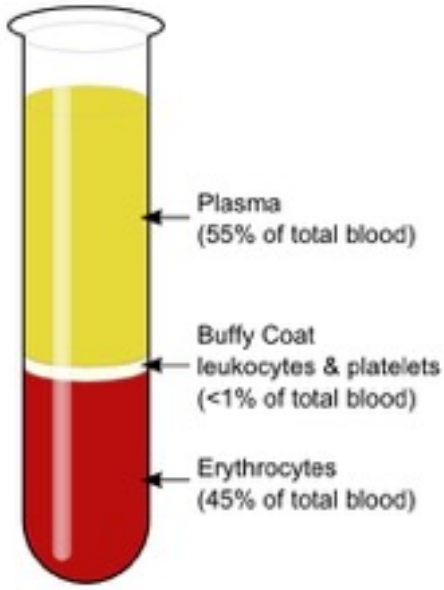
The challenge of precision medicine is to develop and choose best available therapies based on actionable, accessible, and validated biomarkers. For example, it is not clear why certain patients respond to specific types of therapies (i.e., PD-1 inhibitors), while others do not.

At each of these stages, biomarker profiling can:

- inform physicians on how best to manage their patients to slow or even stop the progression of disease.
- help companies developing future treatments, by finding clinically actionable blood-based biomarkers that can segment a disease population, to improve response and get to the market faster.

Blood is the best vehicle for biomarker information

Blood is the body's vehicle for the accumulative evidence of pathological insults for diseases. Secreted proteins, extracellular vesicles, and circulating blood cells mediate individualized homeostasis via intercellular communication, immune responses, vascular and endothelial cell function, tissue remodeling, fluid exchange, and nutrient assimilation. Thus, most diseases are multi-factorial with many proteins collectively acting within highly regulated networks – even single gene diseases can be at the center of larger, complex regulatory networks.



Proteomic information can be derived from all blood compartments

>Plasma/serum proteins and other circulating factors directly regulate complex processes such as aging, the development of chronic diseases, and severe acute disease (i.e., acute respiratory distress syndrome).

>Activated leukocytes and platelets release granulocytic cargo proteins in response to local inflammatory stimuli, altering steady state homeostasis, contributing to both acute and chronic disease.

>Erythrocytes do not have a nucleus and carry more than just oxygen. Red cells have a different make-up of complement regulators than nucleated cells and can lead to dysfunction. Erythrocytes are under investigation for many chronic conditions including Parkinson's Disease and Malaria.

BSG's products and methods can help proteomic investigators explore all these blood compartments.

A disease state results whenever this protein network becomes dysregulated via a confluence of infectious pathogens, heredity, lifestyle, or environmental stimuli.

- ➢ Blood mediates coordination between nonadjacent tissues, so it is essential to understand how this dysregulation manifests itself, regardless of the underlying causative factors.
- ➢ Quantitative proteomics from blood can help unravel these regulatory elements.

Yet, extracting and characterizing functional changes and adaptations to disease for many of even the highest abundance proteins in circulation remains limited.

Proteins are the ground truth of biology, but proteomic productivity can be improved

Proteomic productivity is not measured by Venn diagrams, but rather by quantitative differences between proteins in samples representing a challenge or disease state, vs. samples representing a normal or control state. However, small biological variances are hard to measure robustly, given the nature of technical variance in LC-MS/MS analysis. Large variance proteins (>2x) therefore are the best choices, but this limits the amount of potential biomarker proteins to small numbers, maybe 10-20 rather than the anticipated 1000's expected when the concept of proteomics was first introduced. Aligned with that need, is also the need to measure protein concentrations across several logs in one analysis.

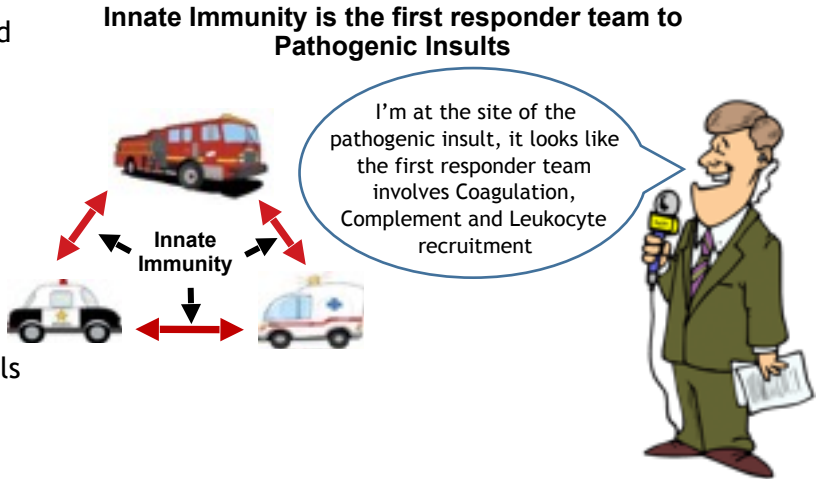
In blood for example, the central Complement protein, C3 circulates at ≈1500 µg/ml, while Complement Factor D circulates at ≈3 µg/ml. To measure both in one analysis requires that the signal intensities at both ends of the spectrum be at least reasonably proportional to the real concentrations, which at times can be across > 4 log ion abundance signal. Thus when selecting potential biomarker proteins, it is best to bias towards proteins in the mid-high abundance range, as low abundance proteins are often subject to signal to noise variance, making them barely detectable and often well beyond the range where signal intensity is proportional to concentration.

The solution demands:

- Fast and efficient sample prep products and methods to allow quantitative monitoring of multiple blood proteins in the mid-high abundance range.
- Enrichment of biomarker proteins from low-abundance to mid-abundance to improve linearity between the measurable peptide ion signals and true protein abundances.
- Strategies for selection of biomarker proteins in the mid-high abundance range which change characteristically with the onset of disease.

BSG has strived to address and service all these demands.

Proteome Categorization distills better information about the innate immune response



The human immune system has evolved to adapt and respond to a variety of physical insults and never-ending exposure to infectious agents to survive. While the emergence of COVID-19 reminds us that infectious insults remain a large healthcare threat, mankind's pharmacological skills (e.g., antibiotics, vaccines, etc.) has largely eliminated many infectious

As a result, we are able to live longer than our predecessors. Unfortunately, our pharmacopeia and inflammatory response systems are not sufficiently capable of fending off today's longer-lifetime exposure to environmental and lifestyle insults that we now face. These exposures and insults contribute to chronic inflammation which, over time, are manifested in a variety of pathological conditions. As witnessed with COVID-19, the pre-existing inflammatory status, may be a key factor to the severity of disease upon exposure to infectious agents.

Innate immunity refers to first-line, non-specific defense mechanisms that come into play immediately or within hours of a perceived pathogenic insult in the body. When functioning properly, it is resolved within 2-14 days. Its main purpose is to immediately prevent the spread and movement of foreign or non-self pathogens throughout the body, and to initiate the second line of defense, the adaptive or acquired immune response. As a second-line defense, the adaptive response (e.g., T Cells) occurs downstream from the innate immune response and starts transferring immunological longer term memory towards the non-self pathogens. So a normal resolution of the innate response leads to a productive handoff to the adaptive response. Conversely, an unresolved innate response may delay or confound a suitable adaptive response, with both acute and chronic disease consequences.

As first responders, the innate immune system is an integrated triangulated network that when functioning properly provides steady state control of pathways involved with coagulation, the Complement cascade, and leukocyte recruitment. Any dysregulation within one affects the others and so collectively, a dysregulated innate immune system can

contribute to the genesis of many acute and chronic pathologies. For example, chronic inflammation is widely recognized as a potential contributor to cancer and many progressive debilitating diseases (e.g., inflammation due to *H.pylori* leads to stomach lesions and cancer). In the clinically severe presentation of the acute respiratory distress syndrome (ARDS), Neutrophil activation and persistent intravascular coagulation as well as impaired fibrinolysis may play a role in the pathogenesis of viral-induced ARDS.

Because the innate immune system has limited differential gene expression over time, protein level orchestration is the differentiator. As we learn how key systemic cross-talk mechanisms may go awry, therapeutically modulating even one rogue component may ultimately unwind the overall dysregulation and contribute to longer term management of disease. For these reasons, we have focused on key regulatory elements of innate immunity that can be observed, reported and surveyed by proteomic analysis of serum/plasma: Neutrophils, Platelets, the Complement cascade, and the protease inhibitor family called the SERPINs.

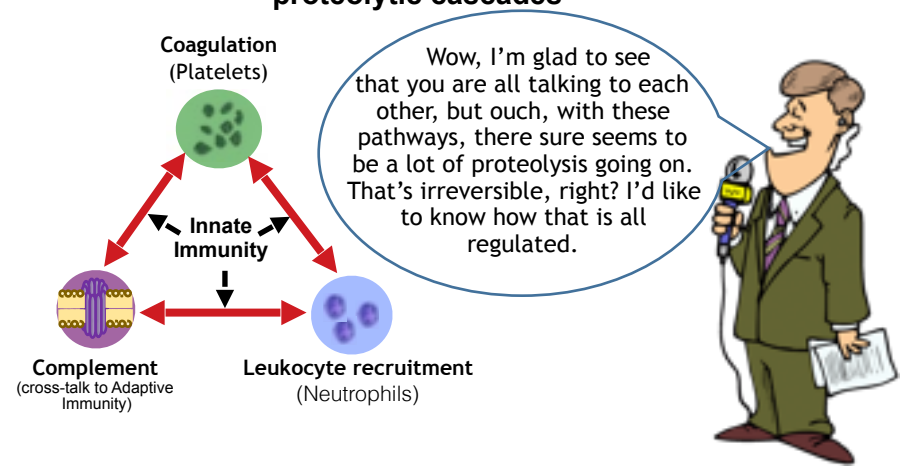
Neutrophils - the most abundant white blood cell, constituting 60-70% of the circulating leukocytes, and they thus contribute to the observable blood proteome much more so than the rest of the white blood cell constituents combined. Neutrophils have extensive crosstalk with each of the major blood cell subsets (megakaryocyte/platelets, myeloid and lymphoid), and release into circulation, cargo proteins from 5 different granule types in coordinated response to inflammatory insults, further magnifying their importance in health and disease.

Platelets - do not have a nucleus, and contain dense and alpha granules; granule secretion being pivotal to establishing and controlling the microenvironment at the local inflammatory site. Platelets release contextually appropriate or kinetically controlled cargo that influences the entire immune spectrum, operating in parallel with their better known clotting function. Whereas rapidly released cargo can mediate early activation necessary for hemostasis, slow or prolonged release can persist long after the initial stimuli. Persistence of Platelet-released along with Neutrophil-released cargo in unresolved chronic inflammation, necessitates a systemic response to regulate the ensuing ‘protease storm’.

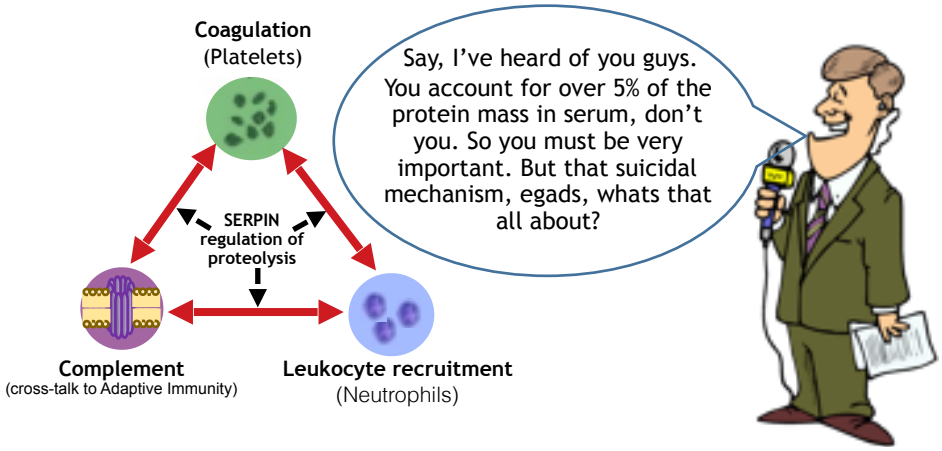
The Complement cascade - a major component of the immune system that provides powerful host surveillance and protection from invading microbes. Comprising about 5% of the total protein mass in plasma, most complement proteins circulate in blood as inactive precursors (zymogens); when triggered, become activated through proteolytic cascades. Cargo proteins from Neutrophils and Platelets regulate Complement activation in a tug-of-war fashion imposing a duality of positive and negative outcomes, in acute and chronic conditions.

SERPINs - a very unique protein family of protease inhibitors. As proteolysis is irreversible, there is an essential balance and regulation of proteolytic cascades necessary to keep competing influences from the triangulated pathways controlled. Central to this maintenance are the inhibitory Serpins, as they collectively serve as the central control function of innate immunity. There are 9 major inhibitory Serpins reportable from blood and collectively account for about 5-10% of the protein mass in serum. However, conventional antigen-based assays fail to report their bifurcated functional pathways. BSG methods can now allow proteomic investigators to observe patterns of 'On' vs. 'Off' Serpin function.

Innate Immunity is interconnected and works through many proteolytic cascades



The Protease Inhibitor family called SERPINs provides a central control function to regulate proteolysis



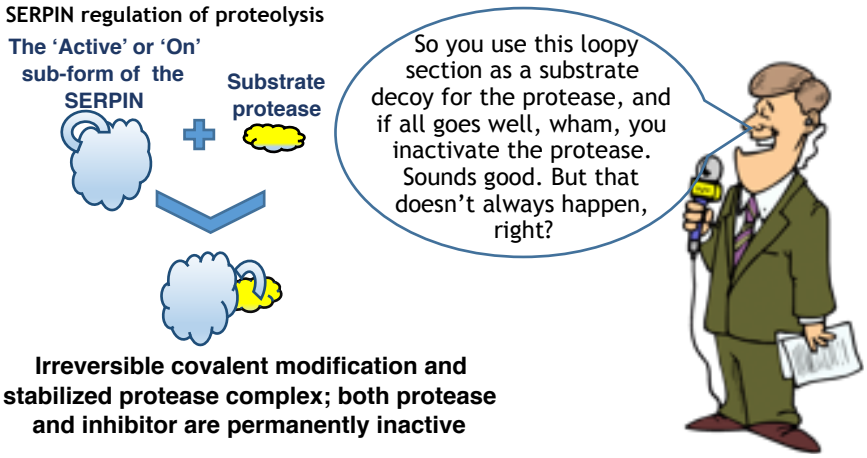
Serpin imbalance with protease activity is well documented in cancer, and used clinically, example: bound vs. free PSA

"The majority of immunologically identifiable human prostate derived proteases, used clinically to monitor patients with prostate cancer, is found in complex with ACT (Serpin A3)"
Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1535.3 (2001): 221-235.

"…a deficiency in α1-antitrypsin (Serpin A1) is associated with increased risk of liver, bladder, gall bladder, lymphoma and lung cancer. "Normally in the general population, the concentration and activity rates of α1-antitrypsin and neutrophil elastase are in balance. It is the imbalance between these two or a net effect of excess neutrophil elastase that might cause various pathological consequences" *The Lancet. Oncology* 5 3 (2004): 182-90

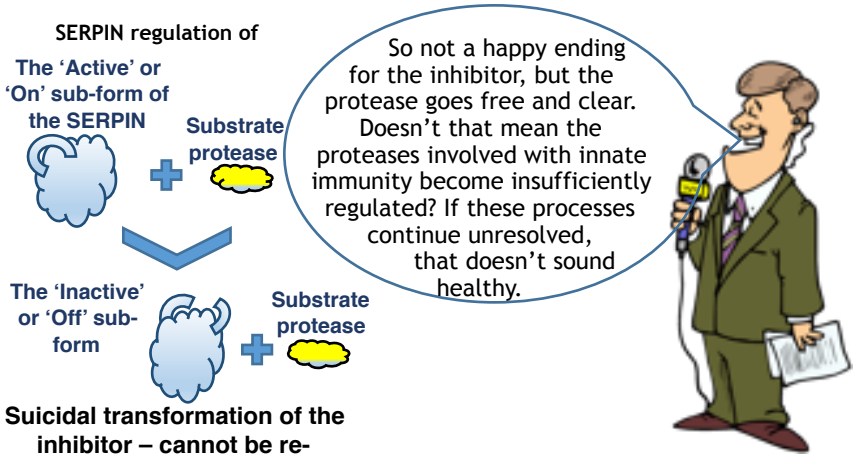
"Under normal conditions, α1-antitrypsin (Serpin A1) is a major antiproteinase… However, **in cancer…a large portion of systemic α1-antitrypsin is biologically not functional**" *CANCER RESEARCH* 44,

SERPINs have a unique mechanism of action



Irreversible covalent modification and stabilized protease complex; both protease and inhibitor are permanently inactive

In chronic inflammation, platelets and neutrophils continuously release proteases which can gradually consume the 'On' Serpins, generating more 'Off' sub-forms



Suicidal transformation of the inhibitor – cannot be re-generated back to active form

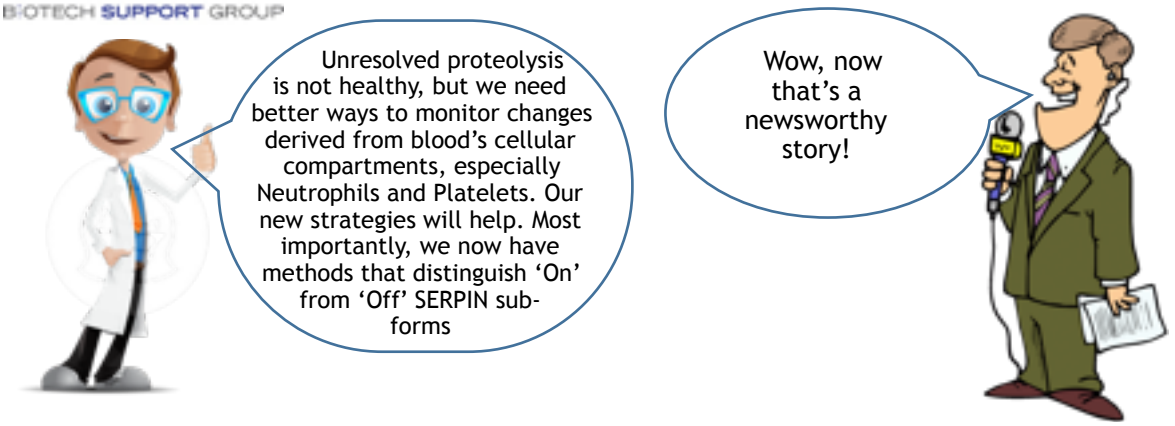
"In a retrospective observational study of 11,116 patients… with suspected SARS-CoV-2, we found that history of macular degeneration (a proxy for complement activation disorders) and history of coagulation disorders… are risk factors for morbidity and mortality in SARS-CoV-2-infected patients...these data suggest that hyperactive complement and coagulative states predispose individuals to adverse outcomes associated with SARS-CoV-2 infection and that deficiencies in complement components may be protective." Nature Medicine (Aug. 2020).

"Significant evidence indicates that a dysregulated innate immune response contributes to the clinical presentation of patients with severe COVID-19 infections." J Exp Med (2020) 217 (6).

"We identified molecular changes in the sera of COVID-19 patients compared to other groups implicating dysregulation of..., platelet degranulation and complement system pathways". Cell 2020.

"In many respiratory diseases characterized by an intense inflammatory response, the balance between proteolytic enzymes (proteases, including elastases) and their inhibitors (proteinase inhibitors) is not neutral. Excess activity of neutrophil elastase (NE) and similar proteases has been reported to cause tissue damage and to alter the remodeling process in many clinical conditions such as pneumonia, respiratory distress, and acute lung injury." CHEST, Volume 152, Issue 2, August 2017.

An Innate Immune Response Proteomic Score Will Provide Very Valuable Characterizations of Disease



Antigen presentation (i.e., immunoassay) has been the basis for SERPIN measurements in the past. With such analyses, all sub-forms are counted as if they are from a singular and homogeneous population. This can lead to egregiously misleading interpretations of how proteolysis is regulated in the general blood circulation.

BSG methods can now differentiate ACTIVE from INACTIVE SERPIN sub-forms using LC-MS/MS, leading to new biomarkers for precision medicine and a greater understanding of disease progression.

"We've looked at blood serum from cancer patients. Our initial functional results support that in the cancer patients, there is a net decrease in inhibitory active Serpin D1, otherwise known as Heparin Cofactor II. Hopefully this brings to light a potential new cancer therapeutic strategy, to inhibit Thrombin in the extravascular space. ", states Ingrid Verhamme, Ph.D., a Serpin expert investigator at Vanderbilt University Medical Center.

That's the power of proteomics, its unbiased data driven analysis.

We highlight detailed strategies to measure the variety of cellular granule cargos, Complement-related and Serpin functional proteins in the journal article entitled "New Strategies to Categorize Blood for Proteomic Biomarker Discovery", see **For more information and additional resources**, at the end of the ebook.

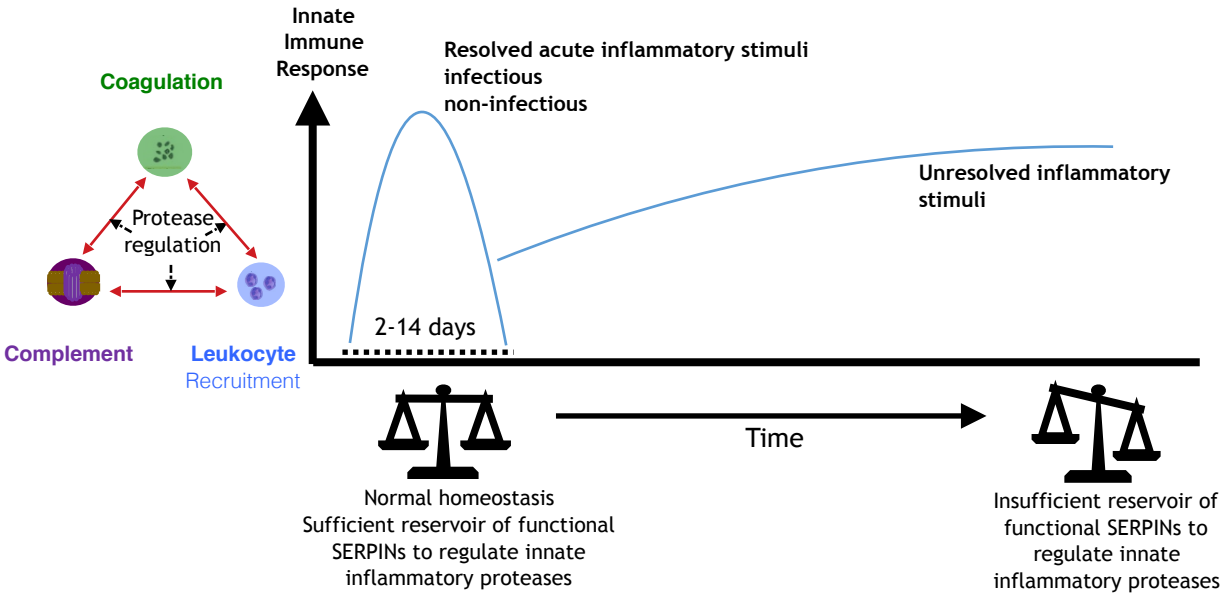
>Most proteins from these blood compartments are highly observable in serum/plasma even with minimal LC-MS/MS acquisition time (= or < 1 hour)

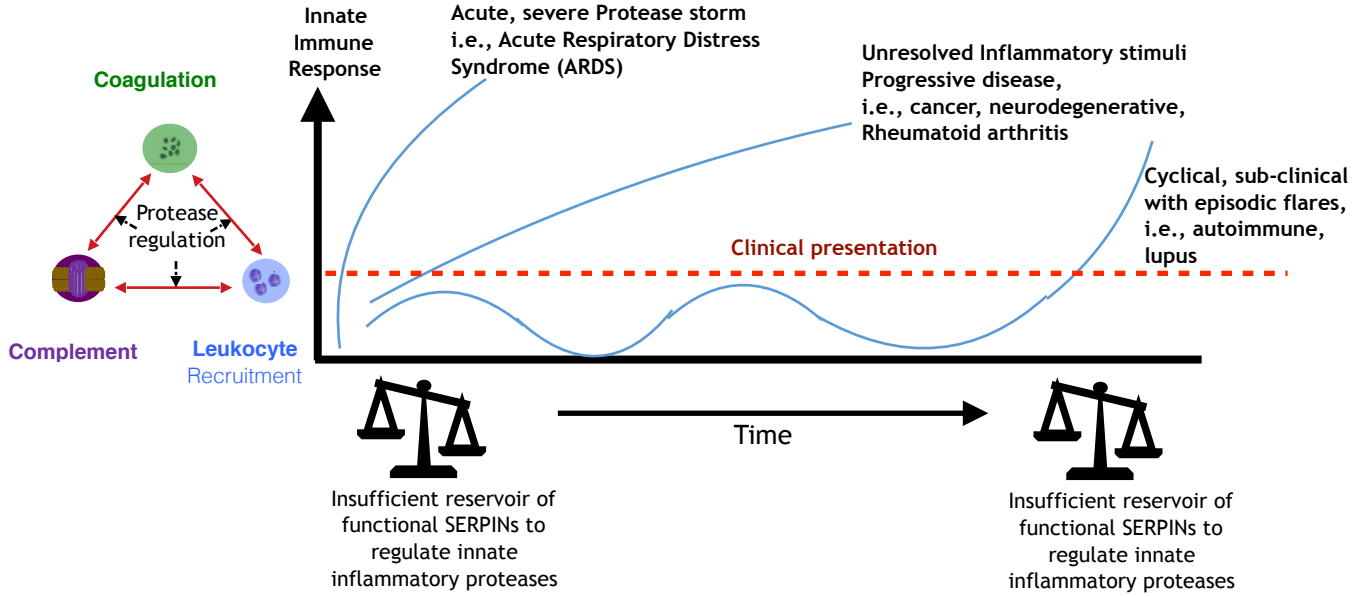
>Most are in the sweet spot (>1 µg/ml) for targeted quantitative LC-MS/MS analysis, especially after enrichment.

>Methods to monitor ACTIVE ('On') vs. INACTIVE ('Off') sub-forms of SERPIN function are described.

Confluence of technologies, methods and strategies will improve proteomic productivity and generate actionable biomarkers

Proposed Model for SERPIN / Protease Balance for Resolved and Unresolved Inflammatory Stimuli





The current clinical practice in laboratory testing for the vast majority of treatment decisions are made on the basis of blood tests, singular protein measurements (i.e., HbA1c) being the most prominent among them. Yet the promise of new singular protein tests derived from proteomics has been woefully unproductive.

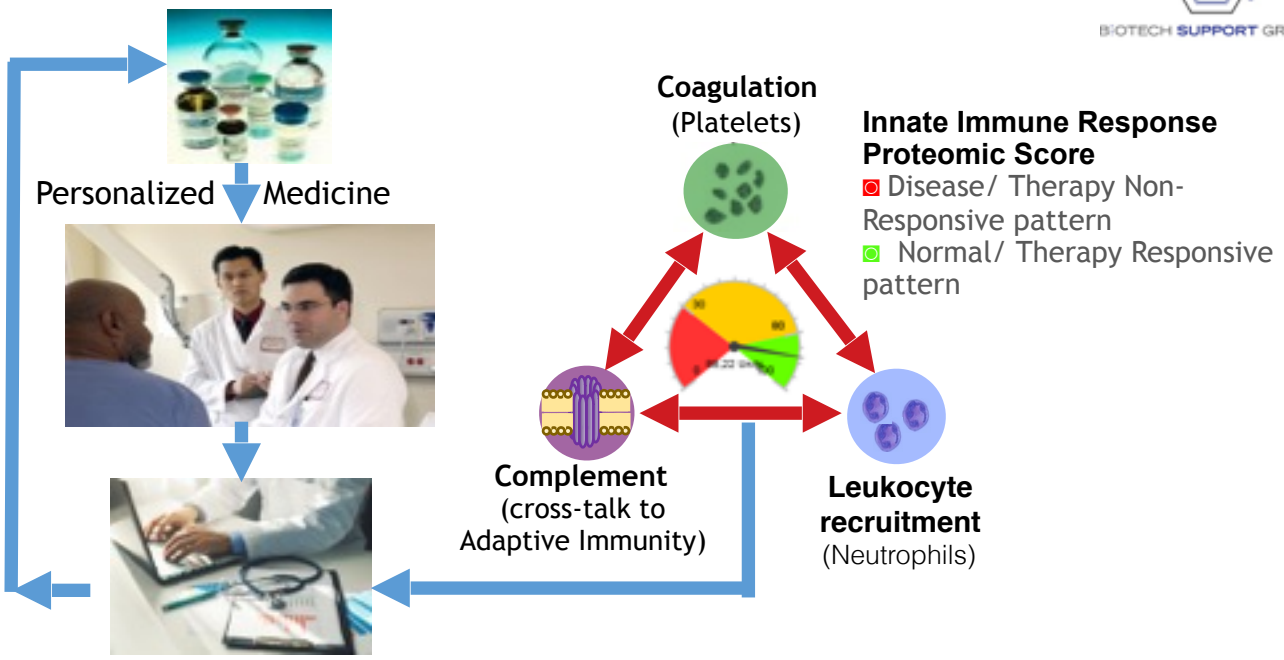
However, unlike single markers such as HbA1c for diabetes management, future advances in protein biomarkers will not come from one protein assay or even a small set of protein assays, but from patterns of protein changes. The good news is that these potential protein patterns are highly observable using LC-MS/MS. Advancements in the quantification of multiple proteins at once, in both targeted label and label-free analysis, coincides with the use of internal standards and simple efficient sample prep workflows.

As a result, targeted LC-MS/MS analysis clearly has the potential for multiplexed and quantitative measurements, in which protein up/down patterns rather than single biomarkers, could be the relevant disease classifier. However, at least for biofluids, practicality would seem to limit the number of multiplexed proteins to somewhere in the 10-20 proteins per test range. Nevertheless, if we focus on the sub-proteome categorization derived from the innate immune response, proxy scores to assess the immune system to the presence of unresolved inflammatory stimuli, or the administration of therapy is now achievable.

As the first line of immune defense, advances in the understanding of the innate immunity proteome in the pathogenesis of pandemic infections, cancer, and autoimmune disease will provide new biomarkers for precision medicine and a resource to re-purpose existing treatments in the future. The convergence of proteomic technologies has made the task immediately available to those who, like us, seek to develop the next generation of molecular tests for more precise and personalized treatment of patients.

Biomarkers that reflect stromal conditioning in tumors will be especially useful to follow the disease response to drug combinations that help to unwind the microenvironments contributing to disease progression.

Upon establishing a joint research agreement with Biotech Support Group, Dr. Wilma Mesker (Associate Professor) and Prof. Rob Tollenaar (Surgeon) of the Leiden University Medical Center state that, “the tumor-stroma microenvironment is an important prognostic parameter for patients with epithelial cancer types. Patients with a high amount of stromal cells in the primary tumor have a bad prognosis and respond worse to current chemotherapy regimens. Blood derived information about various tumor environmental factors could reduce under and over-treatment of cancer patients with chemotherapy, and offers unique possibilities and insight for monitoring during treatment and personalized therapy”.



"Many of the COVID-19 cases I see in the hospital, often first present with bacterial infections, but many go on to a hyper-inflammatory phase, with both coagulation and acute phase biomarkers changing dramatically. At this phase, we introduce anti-coagulants which do appear to help many patients, so therapeutically modulating the innate immune response is a viable approach in my opinion. However, the clinical blood markers we have, for example CRP and D-dimer, while useful to guide therapy, are still very non-specific. A panel of proteomic biomarkers that can more fully characterize the innate immune response would be exceedingly useful to better segment patient populations so we can tailor current and future treatments. Proteomic biomarkers from blood can unlock that future goal we all strive for, and that is personalized medicine."

Devjit Roy, MD FAAFP CHCQM

Hospital Medicine

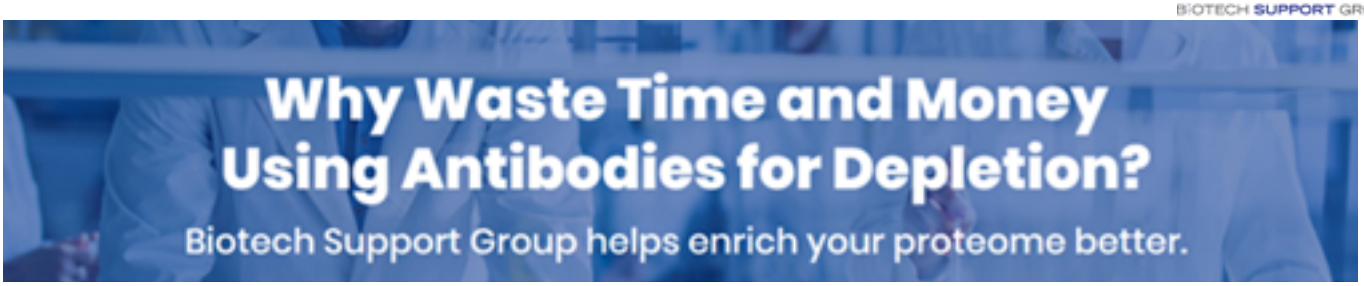
Montefiore-Nyack Hospital, Sound Physicians

Efficient Sample Prep Enrichment Brings Better information from Blood

We've described the need and purpose for quantitative monitoring of multiple blood proteins in the mid-high abundance range. Through reduction of background noise, enrichment of low-abundance biomarker proteins improves linearity between the peptide ion signals and protein abundances. A consumable sample prep product line, not reliant on immuno-affinity, provides the efficiency necessary for clinical proteomic biomarkers derived from blood.

BSG offers a choice of two separation strategies for enrichment, to get the best results: either selectively binding high abundance proteins (the "Bind" products), or choosing not to bind (the "Void" products) thereby enriching the underlying proteome. To achieve this complementary product line, we first developed a chemical library of general non-specific adsorbents, or stated another way - beads with weak affinity or imperfect fit interactions. Without the use of antibodies, progressive displacement at or near saturation, allows the beads to bias for or against certain proteins. After, each product was empirically characterized to meet the needs of the application. The "PLUS" products include a seamless integration of immobilized Protein A, to deplete IgG, a heterogeneous family of hard to digest proteins; this reduces signal interferences across the full LC gradient. With many options, the final product/method choice often depends on workflow considerations and targets to be measured.

- BSG's enrichment products have proven to be robust, reproducible and quantitatively linear across >4x log of LC-MS/MS signal intensity data.
- BSG's unique Bead Assisted Sample Prep (BASP™) digestion protocols minimize inconsistencies of proteolytic digestion, greatly simply workflows, and can often speed the time to analysis.



“AlbuVoid™ method proved to be faster and more cost-effective than antibody-based methods”. Current Topics in Peptide & Protein Research 19. 53-62.

“The advantage of HemoVoid™ in detection of low abundance proteins can be seen when comparing their amounts (in percent) between HemoVoid™ and the other four extraction conditions.… Most peptides, following HemoVoid™, showed ion abundances ranging between 1.00E+5 and 1.00E+6 (31%). In comparison to this, fewer peptides (10–23%) were within this range following extraction with all other protocols.”. Analytical and Bioanalytical Chemistry Feb. 2020

“To obtain purified exosome fractions for proteomic analysis,… albumin was depleted … using AlbuSorb™ - Albumin Depletion Kit” Scientific Reports 8.1 (2018): 7227

“Reticulocytes were lysed…HemogloBind™ suspension was added to the samples, …The supernatants, which contain hemoglobin-depleted sample, were … processed for TMT quantification.” Science 357.6350 (2017)

”If the elimination of lipids…is necessary, the sample can by treated with lipid removal (Cleanascite™)…” Proteomics for Biomarker Discovery. Humana Press, New York, NY,

The BSG Advantages

Cost Effective & Efficient

Sample prep methods essential for expanding proteomic biomarkers into routine healthcare

Consumable Research Products

Supporting the expanding installation of LC-MS instruments & computational infrastructure

Serves All Proteomic Analytical Platforms

Mass Spectrometry (LC-MS/MS, MALDI), Immunoassays, ELISAs, Western blots, 1 & 2 DE, Enzyme & Functional Assays

Species Agnostic

Not derived from immune-affinity, all products work for all species

Knowledgebase of 1000+ Serum Proteins

Supports targeted & quantitative protein markers from serum/plasma

Selection Criteria For Proteomic Sample Prep and Enrichment Products for LC-MS/MS

	Albumin & IgG Removal		Hemoglobin Removal		Lipid Removal
	AlbuVoid™ PLUS	AlbuSorb™ PLUS	HemoVoid™	HemogloBind™	Cleanascite™
Discovery LC-MS	Recommended for Serum	Recommended for Serum	Recommended for Red Cells, Whole Blood and Dried Blood Cards	Recommended for Red Cells, Whole Blood and Dried Blood Cards	Depletes Lipid associated proteins
Quantitative Targeted SRM/MRM	Recommended for Serum May be target(s) specific	Recommended for Serum May be target(s) specific	Recommended for Red Cells, Whole Blood and Dried Blood Cards May be target(s) specific	Recommended for Red Cells, Whole Blood and Dried Blood Cards May be target(s) specific	Depletes Lipid associated proteins
Innate Immune Response Scoring (requires Total Complement Enrichment)	Recommended				
Low Abundance Serum Proteome Enrichment (Complement depleted)		Recommended			
Bead-assisted Sample Prep (BASP™)	Recommended		Recommended		Suitable, but not yet validated

For Albumin & IgG Removal Kits, visit

https://www.biotechsupportgroup.com/Albumin-Removal-s/307.htm

For Hemoglobin Removal, visit

https://www.biotechsupportgroup.com/Hemoglobin-Removal-s/312.htm

For Lipid Removal & Clarification, visit

https://www.biotechsupportgroup.com/Articles.asp?ID=456

For more information and additional resources:

Kuruc M, Zheng H, Sowerhardy A, Avadhani S, Roy D, et al. (2020) New Strategies to Categorize Blood for Proteomic Biomarker Discovery. Proteomics Bioinformatics, 2(2): 90-107.

Although much effort has gone into genomic sequencing to define disease, the downstream products of gene sequences-proteins, nevertheless remain the master regulators of biology via their interactions with nucleic acids and other macromolecules. Many proteins are measurable in blood, making it a rich resource for biomarkers. Yet for reasons largely unrelated to analytical limitations, this resource remains largely untapped. In this review, we describe how chronic illness manifests itself in blood and how we might study innate immunity to understand mechanisms that can potentially translate into new biomarkers and therapeutic modalities. We draw upon our own knowledgebase of proteome information reportable after using depletion or enrichment products in LC-MS/MS workflows and how this knowledge can be utilized in new strategies for biomarker discovery from blood samples. We note that BSG's products have simply and efficiently reduced the complexity of the serum proteome allowing for cost-effective workflows, without the use of antibody-based depletion methods. Finally, we discuss how patterns of Serpins, a superfamily of protease inhibitors, may serve as a surrogate measure of the progressive stages of the innate immune systems' response to both infectious and non-infectious disease. This convergence of strategies and LC-MS/MS technologies has made the task immediately available to investigators to now develop the next generation of molecular tests for more precise and personalized treatment of patients.

This poster was presented at The Serpins2019 Conference, September 19-22, 2019 in Sevilla, Spain, and entitled "Loss of Functional Alpha-1-Antitrypsin and Heparin Cofactor II in Inflammation and Cancer". Authors were: Ingrid M. Verhamme, Vanderbilt University Medical Center; Nashville TN, Swapan Roy, Sowmya Avadhani, Matthew Kuruc, Biotech Support Group LLC, Monmouth Junction NJ.

The poster describes that in various cancer types, blood serum levels of Serpins have been reported as altered. Significantly however, is that in these reports, concentrations were measured by immunological methods (ELISA) rather than by functional activity. This can lead to egregiously misleading interpretation of a host's systemic response to cancer, as the immunological assay measures in aggregate both the intact and cleaved Serpin forms. In contrast to these methods, we demonstrate that with albumin depleted (AlbuVoid™) serum samples, combined with LC-MS/MS, there is potential of distinguishing between active and cleaved subpopulations of Serpins. Cleaved Serpins are the product of the substrate pathway in the bifurcated Serpin suicidal mechanism, which can become more pronounced due to Serpin mutations or changes in the micro-environment.

Zheng et al., "AlbuVoid™ Coupled to On-Bead Digestion - Tackling the Challenges of Serum Proteomics". J Proteomics Bioinform 2015, 8:9 DOI: 10.4172/0974-276X.1000373

Using 2 different allotted digestion times - 4 hours, and overnight, each with a singular 3 hour gradient LC-MS run, between 400-500 total proteins were observed for both human and rat sera, with mostly overlapping but also with distinct sub-populations observable at each digest time. These results support that the described methods gain efficiencies over antibody depletion and in-solution digestion workflows, for both discovery and quantitative serum proteomic applications.

Application Note entitled "AlbuVoid™ PLUS & AlbuSorb™ PLUS - Evaluating Different Windows of Observation Solves The Many Challenges of Serum Proteomics"
https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/PLUS%20Application%20Report%2007212019%20v1.pdf

For serum, many proteomic enrichment strategies employ the use of immuno-affinity depletion to remove one or more high abundance proteins. Some common limitations of immuno-affinity however are high costs, and regeneration requirements which may result in a diminished and inconsistent performance. Because of these limitations, proteomic researchers need ways to enrich without immuno-affinity. This report considers the advantages of first reducing the influence of IgGs- a heterogeneous and proteolytically resistant class of proteins, along with Albumin depletion. Two products - AlbuVoid™ PLUS & AlbuSorb™ PLUS support depletion of both Albumin and IgG, through different strategies and workflows. Using LC-MS reporting metrics, the report highlights the serum sub-proteome bias characteristics of these products. Some examples of their selective utility for biomarker discovery in cancer are also presented.

Case Study entitled “Establishing the Utility of HemoVoid™ and HemogloBind™ as Enrichment Tools for Proteomic Analysis of Red Cells and Whole Blood in Parkinson’s Disease

The complex etiology of Parkinson’s disease (PD) is only poorly understood. However, growing evidence now suggests that neurodegeneration in PD is not restricted to dopaminergic neurons in the brain. Rather, PD may be a systemic disease, involving peripheral tissues and may include oxidative, metabolic, or inflammatory processes. So blood based biomarkers may prove useful in early detection of PD as well as to assess the progression of disease in response to medical interventions.

Whitepaper entitled “Stroma Liquid Biopsy™ - Blood-based biomarkers to monitor stromal conditioning in cancer.” Published February, 2019.
http://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/StromaLiquidBiopsyWhitepaper022519.pdf

The whitepaper describes that tumors are more than simply a collection of immortalized cells as the supporting microenvironments or stroma also contributes to pathogenesis. Because of this, tumor characterization cannot be fully characterized solely through the analyses of the tumor cell genome – the current emphasis of liquid biopsy platforms. So because tumors are more than just a mass of proliferating cells, cancer progression must take into consideration the influence of the multiple cell types and networks of host response proteins dynamically interacting in active tumorigenesis. These are not simply passive bystanders. The unique significance of the Stroma Liquid Biopsy™ pan-cancer profile is that dysregulation in blood was categorically intertwined with the most rudimentary needs of cancer: space, nutrients and immune evasion. Moreover, the changes within the 13 biomarker panel all occur within an interdependent network of cascading proteolytic events. Because proteolysis is irreversible, all species of life have evolved molecular regulatory systems to control aberrancies; the most distinguished is a protease inhibitory family of regulators known as SERPINs.

Chapter from Book: Functional Proteomics – Methods and Protocols, publisher Springer 2018. “Methods to Monitor the Functional Subproteomes of SERPIN Protease Inhibitors”.

https://www.biotechsupportgroup.com/v/vspfiles/templates/257/pdf/FunctionalProteomicsBookSERPINChapter.pdf

Conformational variants of the unique family of protease inhibitors annotated as SERPINs are most often under-represented in proteomic analyses. This limits understanding the complex regulation that this family of proteins presents to the networks within the protease web of interactions. Using bead-based separation provided by a family of proteomic enrichment products—notably AlbuVoid™ and AlbuSorb™, we demonstrate their utility to satisfy investigations of serum SERPINs. We also suggest their use to develop functional profiles of the SERPIN proteoforms, and how those can establish relationships to disease phenotypes, gene mutations, and dysregulated mechanisms.

About Biotech Support Group LLC
Converging with cultural and technological disruptions forthcoming in healthcare, Biotech Support Group develops methods for cost effective and efficient sample prep essential for these expanding markets. Following a tiered business strategy, the company continues its growth in the consumable research products area supporting the rapidly expanding installation of LC-MS instrument and computational infrastructure. From these innovations, the company has acquired knowledgebase and biomarker intellectual property assets that support discoveries of protein markers from blood, with special emphasis on early detection and personalized medical decisions for cancer patients. For Business Development, contact: Matthew Kuruc 732-274-2866, sales@biotechsupportgroup.com

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