

Review Article

Identification and Validation of a Prognostic Biomarker for Septic Arthritis: A Hypothesis

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ABSTRACT

Inflammation is a hallmark of many diseases including septic arthritis (SA) or infectious arthritis (IA). The bacteria *Staphylococcus aureus* is the most common cause of septic arthritis in humans. The statistical incident rate for SA is 2-10 per 100,000 patients years in the USA and Western Europe. Currently, there is no ideal diagnostic or prognostic biomarker for SA at the moment. By virtue of their lack of specificity and accuracy, traditional inflammatory biomarkers are often ineffective in distinguishing SA from infectious joint disease. The present hypothesis wishes to explore the discriminatory power of unique inflammatory biomarkers in liquid biopsy samples of bacteria induced mice and SA patient samples by means of genome wide analysis in which includes epigenetic, genomics and transcriptome expression pattern. Investigation on gene expression by RNA sequencing and DNA methylation studies provides the best possible target genes of differentially expressed both RNA and DNA marker genes. The aim is to find a meaningful positive or negative association between gene expression in RNA sequencing data and DNA methylation status. Validation of differentially expressed genes and differentially methylated genes by functional mechanistic studies to determine the best predictive and prognostic marker for SA for regular therapeutic use by comparison with established reference genes. With respect to bacterial mediated mice vs SA patient gene contrast, the predicted results clarifies the in-depth understanding of pathophysiology, disease development, and SA status.

BACKGROUND

Inflammation is a major culprit for many infectious diseases and is nothing but an non-specific immune system defense against harmful stimuli in human beings which is caused by several factors such includes pathogenic organisms, damaged cells, and irradiation or toxic substances [1]. Inflammation serves as a protective mechanism against damage or illness, as well as the excretion of the original source of cell or tissue injury, clearing out injurious factors induced by trauma, and triggering the healing process through a disrupted homeostasis mechanism [2, 3]. Inflammatory diseases are a broad category of illnesses and diseases characterized by inflammation [4]. For instance, following diseases like autoimmune diseases [5], asthma [6], Crohn's disease [7], tuberculosis [8], chronic obstructive pulmonary disease (COPD) [9], chronic peptic ulcers [10], arthritis and other joint diseases [11] are notable inflammatory diseases. Septic arthritis, also known as infectious arthritis, is an autoimmune joint disease caused by a variety of infectious pathogens. Direct sharp injury or, more generally hematogenous seeding at the vascular synovial portion of the joints triggered by the bacteremic episode results in SA [12, 13]. Since there is no determining cellular plate under the vascular synovial section,

pathogens easily infiltrate the joint during sepsis [14]. Similarly, joint injury can helps bacterial inoculation, so extracellular matrix (ECM) proteins formed at inflamed joints can minimize bacterial adhesion and help infection progress [14]. Overdue or ineffective treatment may result in permanent joint damage [15]. SA has been related to mortality in particularly poorly treated cases, in addition to the illness associated with chronic joint damage [16, 17]. For this reason, there is a need of high specificity and sensitive prognostic biomarker is required to diagnose SA at early stage and begin the treatment quickly.

Epidemiology

The long-term pervasiveness of SA in patients who demonstrated with a severely painful and swollen joint [18, 19]. It presents a serious morbidity and fatality. The statistical prevalence of SA is reported to be 2-10 per 100,000 patients years in the United states and Western Europe [20, 21]. Interestingly, the literature survey showed that SA of a native joint is present everywhere at knee joint followed by the wrist, ankle, hip, shoulder, sternoclavicular joint and elbow [22, 23]. However, SA of the elbow is thought to be

responsible for 3 to 9% of all SA cases reported [12, 24]. SA is normally monoarticular, although in a limited number of cases (around 20%), it may be oligoarticular [19]. Age over 60, existing joint conditions, bacteremia, corticosteroid use, rheumatoid arthritis (RA) prescriptions, skin fragility, a weakened immune system, and degenerative joint trauma are only a few of the risk factors for SA [12, 14]. The prevalence of SA is 10-fold greater in patients with RA as compared with normal population [22]. Disease-causing bacteria are reposed at the synovial membrane during the process of a septicemia, which occurs from a portion of an external joint, and in extreme cases accumulate to cause a SA [25]. Cellular pathology of a SA make a permanent cartilage damage in an intricate joint and it can happen within 8 hours of infection that may also associated with collateral damage, stab wounds, and intra-articular injections [26]. The highly vascularized joint synovium lacks a restrictive basal lamina, making it susceptible to contagion through hematogenous seeding from long-term infection [27, 28]. Infection causes inflammatory cells (polymorphonuclear leukocytes) to release proteolytic enzymes, which demolish the mucopolysaccharide base material of articular cartilage and allow the collagen fibers in the cartilage to begin to be ruined by frictional mating of the joints during movement [29].

BACTERIOLOGY

Until 1980, *Pseudomonas aeruginosa* was the most common pathogen responsible for SA, but now Gram-positive bacteria such as *Staphylococcus aureus* (*S. aureus*) (56 percent) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) account for the vast majority of cases (approximately 10-20 %)[12, 30-32]. Despite the fact that any pathogen can cause SA in humans, *Streptococcus pyogenes* is the second most prevalent cause after *Staphylococcus aureus*, which is most often linked to trauma, chronic skin infections, and autoimmune disorders [12, 22, 33].

CLINICAL PRESENTATION

According to Margaretten et al., indicated a SA carries five different cardinal signs such include pain (85%), swelling (78%), redness (19%), heat (46%) and loss of function (27%) [34]. Regardless, a large number of patients may present with very amiable disease manifestations, especially when the immune system is compromised, which may result in a late diagnosis [35]. Greater attention have to be given to RA patients in one inflammatory burn, as it might be virtue of being fundamental prerequisite and not only that to overlying SA [12].

DIAGNOSIS AND BIOMARKERS

The general diagnosis for SA is followed by routine tests that include A) physical examination (inspection, palpation and motion), B) imaging studies (plain radiographs, ultrasound and MRI), C) laboratory studies includes a) blood serum analysis (estimation of C-reactive protein levels), b) Joint fluid aspiration (characterized by differen-

tial white blood cell count, Gram staining, culture, glucose level and crystal analysis) and c) saline load test (to check the joint sensitivity) [36-42]. According to current research, prompt surgical action will help mitigate long-term joint damage [12]. Clinicians are now diagnosing SA with traditional inflammatory markers such as CD64 and procalcitonin (PCT). However, the specificity and sensitivity of these markers are limited [43]. Clinicians have a strong demand for the discovery of a perfect high precise and adaptive prognostic SA biomarker for early detection, and it is a must.

HYPOTHESIS

To understand the etiology of SA and identification of novel prognostic marker for the death, survival and remission. There is no perfect prognostic predictor for SA at this time. However, the currently used markers have lacking the specificity and stativity. We will identify the best candidate genes using quantitative simulation and liquid biopsy [44]. To determine the best biomarker genes and disease progression, comparative genome review must be performed on liquid biopsies from *Staphylococcus aureus* induced inbred mice and SA patients. The role of prognostic marker can be determined by studying its pathology, characterizing by means of genome wide analysis in which includes epigenetic, genomic and transcriptome expression pattern.

The major expected accomplishments include

1. The first and most important goal is to obtain blood samples from mice (both normal and bacteria-induced) and patients in order to study the phenotypic and molecular agents or biomarkers associated with SA patients.'
2. Transcriptome profiling and sequencing of over expressed genes in SA. Identification of methylated genes and their impact in altering epigenetics of SA. Possible role of microelements in disruption of extra cellular matrix and invading the neighboring tissue.
3. Creating the genomic and proteomic library for comparative analysis to see the significance and identification of appropriate biomarker for targeting SA in humans for new therapeutics.
4. Functional mechanistic studies of differentially expressed genes and differentially methylated gens: correlate them to see either positively dependent or negatively dependent.
5. Standardization of bioassays for functional mechanistic studies on identified targets in both RNA sequence profiling and DNA methylation profiling for identification of novel diagnostic and prognostic markers for targeting SA as a therapeutic measure.
6. Identifying the disease mechanism of *Staphylococcus aureus* in disruption of extra cellular matrix and invading the vascular synovial membrane and aggressiveness of human SA.
7. Define the unique signature of prognostic marker in human SA.
8. Histopathological and immunohistochemistry for diagnosis of SA.

EXPERIMENTAL DESIGN

Animals

To test the hypothesis, a traditional inbred mouse (roughly 12-20) and peripheral blood samples from Septic arthritis patients (12-20) are required.

Sampling

As seen in Figure 1, blood samples are taken from normal mice, pathogen-induced mice (5th and 10th days), and SA patients. Further, blood samples were split into two sections, one for complete RNA isolation (Qiagen micro RNA kit) and the other for DNA isolation (Qiagen DNA methylation kit), and the downstream study was carried out as seen in Figure 2. The standard paired-end protocol is used to sequence the whole genome on the Illumina GAIIx network. For clinical diagnosis, microbial diagnosis, hematological analysis, and serum protein analysis, synovial samples from Septic arthritis patients and pathogen induced mice are obtained. Complete RNA is isolated for RNA sequencing [45] and DNA is isolated for methylation experiments to determine the best candidate genes in each sample [46].

Bioinformatics and Statistical Analysis

RNA-sequencing data demonstrates the transcriptome profile, which genes are turned on in target samples, level of differential gene expression and what times they have activated or turn off (Figure 2). It also provides us with quantifiable evidence and a low background signal (experimental noise).

This critical knowledge helps one to grasp the disease’s present state [45]. DNA methylation (bisulfite sequencing method) and pyrosequencing are used to determine the total improvements in DNA methylation status as well as how much of the gene/regulatory region had been differentially methylated (Figure 2) [49]. Pyrosequencing can provide objective results, and the procedure is accessible even though there is a slight variation in methylation. RNA sequence raw data is analyzed using bioinformatics methods, which include precise mapping (genome, transcriptome, and reference-free assembly), quantification of differentially expressed genes in various biological settings, and analysis [50-52].

When comparing RNA-sequencing results with DNA methylation using the methylated DNA antibody-based immunoprecipitation (MeDIP) microarray procedure, we will determine the importance and likelihood of the best target genes [53]. Based on the findings, functional mechanistic studies of differentially expressed genes and methylated genes are performed using the methods (CRISPR/Cas9 or siRNA, western blot, and qPCR) to confirm the target genes *in-vitro* or *in-vivo* studies [54-60].

DISCUSSION

Infectious arthritis/SA is a rare disorder with a high rate of severe illness and death, particularly in the elderly [61]. SA was once thought to be a condition that mostly affects individuals above the age of 60 and young girls (under 16 years) [62]. According to the published literature, the knee is the most often affected joint among the other joints [63]. *S. aureus* is the most prevalent microbe in adult joints, affecting people of all ages

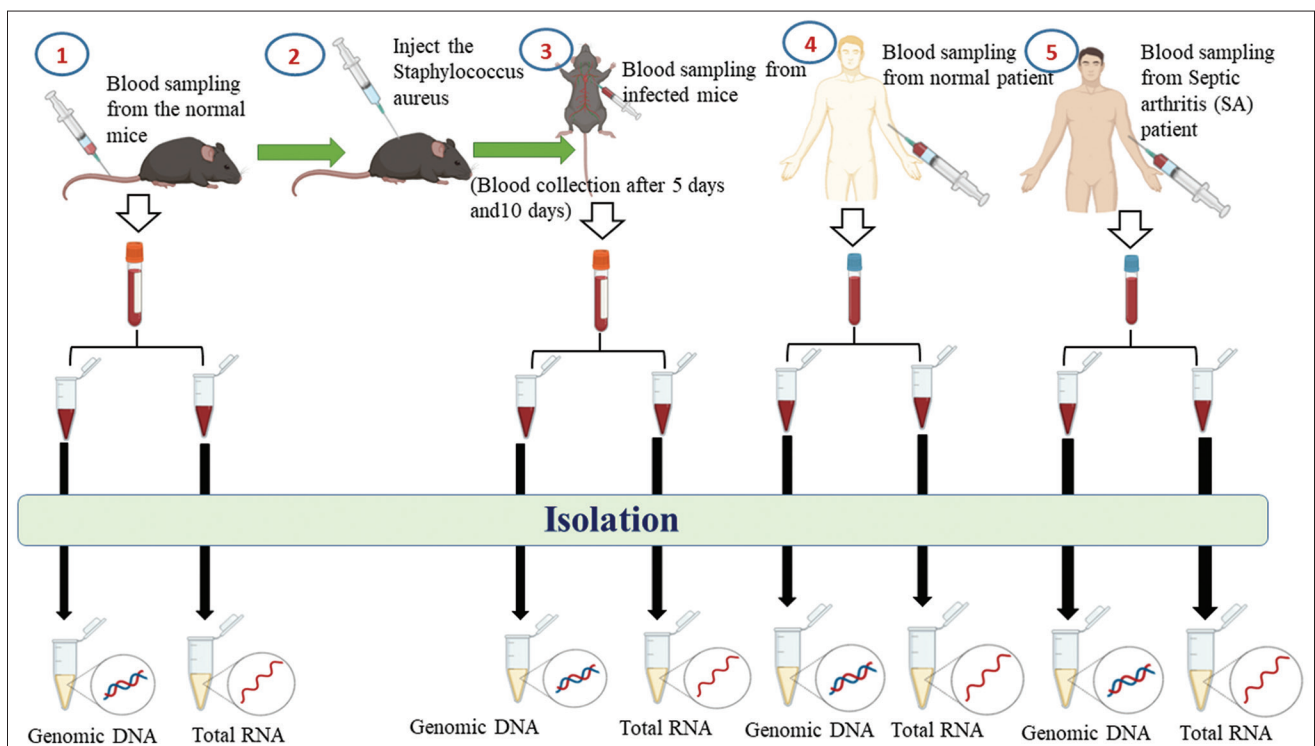


Figure 1. The schematic work plan of the objectives that are most commonly examined in the experimental models in biomedical applications. Isolation of blood from the models in different conditions as depicted in the picture (1 to 5). The figure is modified from [47]

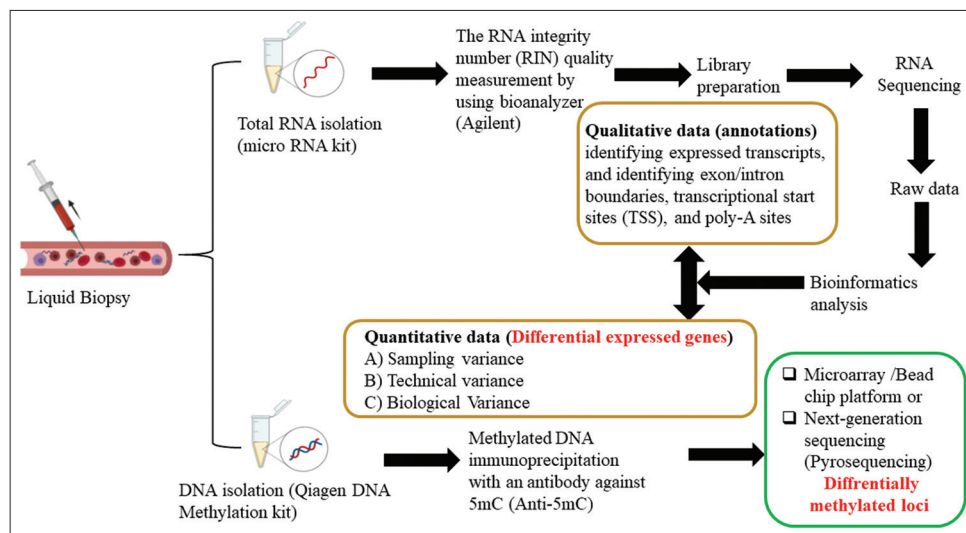


Figure 2. Proposed working procedures to investigate diverse objectives concerned to the domestic mice and septic arthritis patients through genomics, transcriptomics and epigenetic tools. The picture is modified from [48]

and disadvantaged classes, followed by other gram-positive bacteria including *streptococci* [62, 64]. The need for better testing instruments to aid in the detection of infectious diseases is currently underway. CD64 and PCT are highly specific biomarkers for pathogenic infections, especially SA, and may be very useful as rule-in biomarkers. While both markers have drawbacks and have low sensitivity in localized pathogens, they do have some advantages [43]. As a result, more accurate, standardized, and prognostic markers are required to replace the existing biomarkers used in standard medical practice in hospitals.

For a few forms of chronic arthritis, the etiology of SA will remain a mystery, and certain unfavorable effects for certain communities are still unknown [43]. The gold standard for identifying prognostic markers for SA is RNA sequencing and DNA methylation analysis. Spearman's association between differentially expressing genes and methylated genes establishes the statistical dependency of two factors, whether they are positively or negatively correlated [65] and also provides either confined conclusion or normal conclusion. Based on the clinical follow-up of the patient profile (patient cohort, systemic data, and clinical data), we can compare known genes to reference genes to find the ideal prognostic marker (high specificity and sensitivity) in a cost-effective and time-consuming manner [65].

CONCLUSION

SA is a potentially virulent state that regrettably does not attempt to ever-present traditionally. The cardinal signs of fever, redness and swollen joints states in the light of SA. No medical check-up judgement cannot exclude the disease status, and serum blood tests should consider for better diagnosis of SA. The most popular diagnostic tests are synovial lactate and microbial culture. However, no perfect prognostic predictor for death, recovery, or remission exists. According to the current hypothesis, molecular biomarkers may be rationally paired with clinical recommendations to eliminate the risk profile of SA patients as a determining factor. Finally, excellent results can be expected if the experiments are carried out according to schedule. Finally,

we can say that research on genomics and transcriptomics may be a fruitful avenue for finding the best prognostic marker genes for identifying SA for potential therapeutic steps.

ABBREVIATIONS

Chronic obstructive pulmonary disease (COPD), Extracellular matrix (ECM), Infectious arthritis (IA), Procalcitonin (PCT), Rheumatoid arthritis (RA), Septic arthritis (SA).

DECLARATIONS

Ethics Approval and Consent to Participate

Not applicable.

Consent for Publication

Not applicable.

Availability of Data and Materials

Not applicable.

AUTHORS' CONTRIBUTION

The whole manuscript has proposed the design of work and written by Ravi Adusumalli.

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