Identifying Inflammatory Gene Expression Signatures for Skin and Soft Tissue Infections

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ABSTRACT

Background: Misdiagnosis of skin and soft tissue infections (SSTIs) due to clinical mimics can result in delay of care, unnecessary antibiotic exposure, and inappropriate hospitalization. Comprehensive screening of inflammatory genes in SSTIs could identify biomarkers to distinguish SSTIs from mimics.

Methods: We performed a search of the MGH James Homer Wright Pathology Laboratories database from 2008-2018 for diagnoses of necrotizing fasciitis, cellulitis, and stasis dermatitis, yielding 103 cases. Diagnoses were verified by chart review and categorized by discharge diagnosis. Three samples from each category, along with three controls from location-matched skin were selected for further study. mRNA isolated from paraffin-embedded skin biopsies was analyzed by Nanostring, with 594 inflammatory genes profiled.

Results: We identified differentially expressed genes between necrotizing fasciitis, cellulitis, and infectious cases (necrotizing fasciitis and cellulitis) compared to non-infectious stasis dermatitis. Differentially upregulated genes in SSTIs included those with known roles in inflammation (CXCR2, IL6, IFI16, TNFRSF1B) and transcriptional regulation (BCL3, MBP). We also identified differential upregulation of genes not previously associated with SSTIs including S100A8, S100A9, MCL1, CD14, and LTF.

Conclusion: We characterized transcriptomic signatures of severe and moderate SSTIs compared to stasis dermatitis and normal skin from the lower extremities. Though limited by small sample size, these data support the utility of a prospective study analyzing outcomes of patients diagnosed with SSTIs based on gene expression signatures. Identifying SSTI-specific gene expression signatures could help differentiate true skin infections from non-infectious inflammatory skin conditions, facilitating more accurate diagnoses and improving patient care.

INTRODUCTION

Skin and soft tissue infections (SSTIs) are one of the most common reasons to seek medical care in the United States. Cellulitis accounts for ~60% of SSTIs, with 14.5 million cases annually in the United States accounting for $3.7 billion in healthcare costs.¹ SSTIs are frequently misdiagnosed...
due to clinical overlap with inflammatory dermatoses (e.g., stasis dermatitis), resulting in delay of effective care, unnecessary antibiotic exposure, and inappropriate hospitalization.2

Misdiagnosis is partially due to an incomplete understanding of SSTI biology leading to ineffective diagnostic strategies. SSTIs are thought to be mediated by toxin-induced inflammatory pathways3; however, published literature lacks comprehensive screening of inflammatory genes in SSTIs. Identifying SSTI-specific gene expression signatures could help differentiate true skin infections from inflammatory mimics, thereby reducing misdiagnosis and broadening understanding of the pathophysiology to identify potential therapeutic targets.

METHODS

We performed a search of the MGH James Homer Wright Pathology Laboratories database from 2008-2018 for diagnoses of necrotizing fasciitis, cellulitis, and stasis dermatitis, yielding 103 cases. Diagnoses were verified by chart review and categorized by discharge diagnosis. Three samples from each category, along with three controls from location-matched skin were selected for further study. mRNA isolated from paraffin-embedded skin biopsies was analyzed by Nanostring, with 594 inflammatory genes profiled.

RESULTS

One hundred thirty-four cases were identified and verified by dermatologist chart review of discharge diagnosis and dermatopathologist review of skin biopsies, yielding 103 cases which were filtered by lower extremity because this location is common for SSTI mimics, resulting in 71 cases (Figure 1). We confirmed necrotizing fasciitis cases with imaging evidence of subcutaneous air and cellulitis cases with positive bacterial data by culture or Gram stain. Cases with concomitant clinical scenarios such as osteomyelitis, immunosuppression, implanted hardware, and neutrophilic dermatoses were excluded. For this pilot study, three samples from each diagnosis were chosen for further cytokine interrogation. Cultured bacteria from these three cases included methicillin-sensitive Staphylococcus aureus and Klebsiella oxytoca. Additionally, three normal skin controls from location-matched skin and agematched cases (+/- 10 years) were selected to account for background gene expression. Slides were reviewed to confirm the histopathological diagnoses in selected cases (RKF).

mRNA from FFPE skin biopsy samples was analyzed by Nanostring, and 594 inflammatory and immunologic signaling genes were profiled. Raw data were normalized to GUSB expression. T-tests were performed between infectious cases (necrotizing fasciitis and cellulitis) and non-infectious stasis dermatitis to identify candidate genes (p < 0.05). A heatmap was plotted with the resulting genes (Figure 2).

Differentially upregulated genes in both necrotizing fasciitis and cellulitis included those with known roles in inflammation (CXCR2, IL6, IFI16, TNFRSF1B), transcription regulation (BCL3, MBP, SOCS3), and complement activation (C1QB, C1QA, CR1). Neutrophil recruitment by cytokines (IL1a, IL1b, IL6, TNF) and chemokines (CXCL1, CXCL2, CXCL5, CXCL8) is thought to be a key component of the immune response in SSTIs.4 We also identified differential upregulation of genes not previously associated with SSTIs.
Figure 1. Flowchart of case selection for small-scale pilot study.
Figure 2. A) Heatmap of differentially expressed genes between infectious (necrotizing fasciitis and cellulitis) vs non-infectious (stasis dermatitis) cases. B) List of differentially expressed genes with associated p-values.

including $S100A8$, $S100A9$, $MCL1$, $CD14$, and $LTF$. $S100A8$ and $S100A9$ are calcium-binding proteins which form an antimicrobial complex secreted by neutrophils during inflammation.$^5$ $MCL1$ is upregulated during phagocytosis to protect macrophages from apoptosis.$^6,^7$ $CD14$ recognizes lipopolysaccharides in bacterial membranes.$^8$ LTF helps in antimicrobial defense by competing with microbes for iron.$^9$

**DISCUSSION**

Here, we characterized transcriptomic signatures of severe and moderate SSTIs compared to a common clinical mimic, stasis.
dermatitis, identifying known and novel genes involved in inflammatory processes and infection. Although limited by its small scale, our study identified additional genes beyond what was previously identified in another transcriptomics study comparing bacterial cellulitis to normal-skin controls. Though limited in sample size, these data represent a pilot study in the understanding if SSTI gene regulation and support the utility of a prospective study analyzing patient immune signatures in conjunction with histology to differentiate SSTIs from clinical mimics. This work could inform innovative strategies that would fundamentally change SSTI management by providing rapid, point-of-care, objective diagnostics. Identification of tissue-specific and systemic biomarkers in patients with SSTIs may allow prognostic stratification to guide choice of therapeutics. Overall, improved diagnosis for SSTIs will help reduce unnecessary hospitalizations, antibiotic overuse, and healthcare costs and complications.

Conflict of Interest Disclosures: None

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