SKIN

BRIEF ARTICLE

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 pathways[3]; 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 age-matched 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

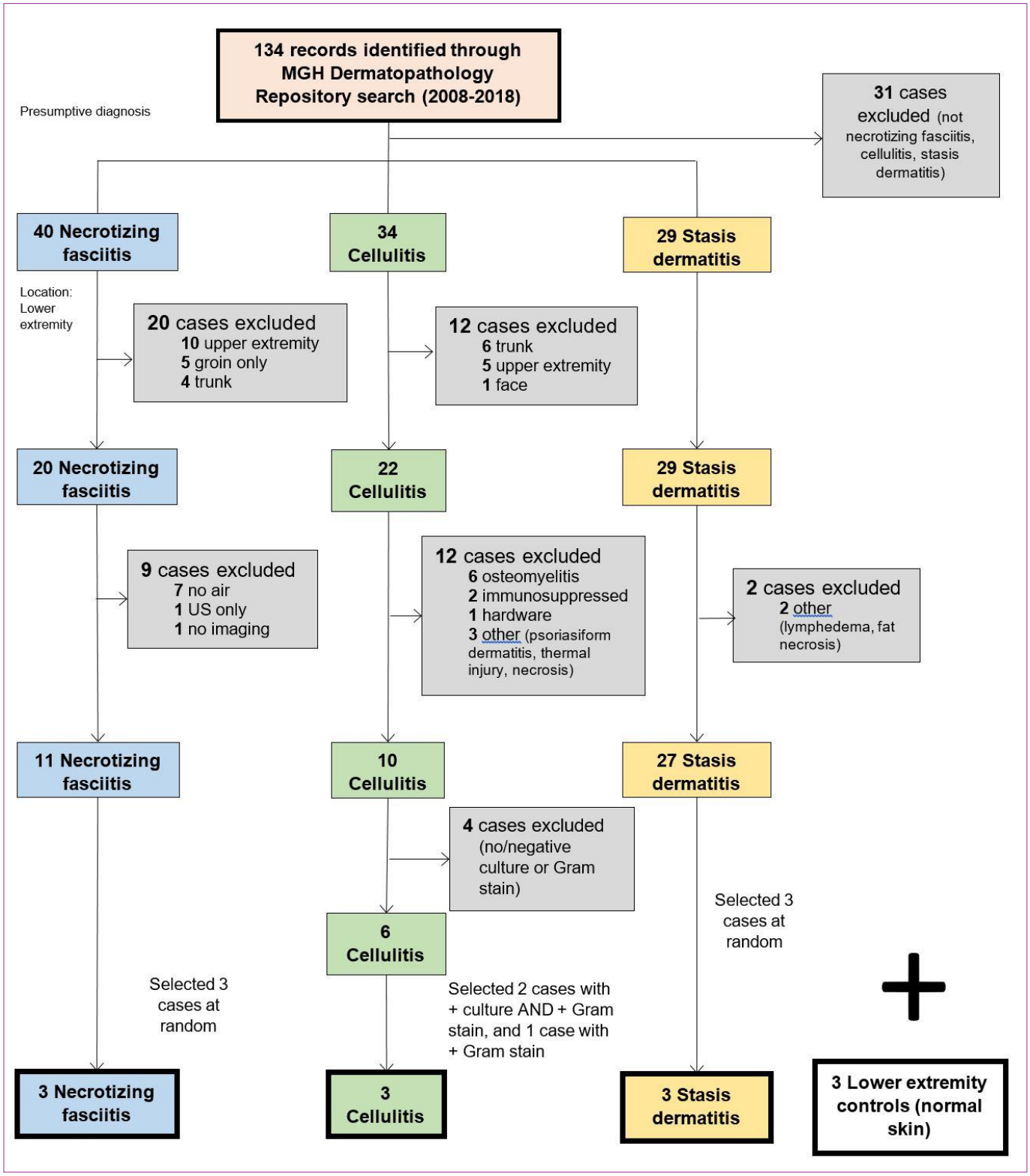
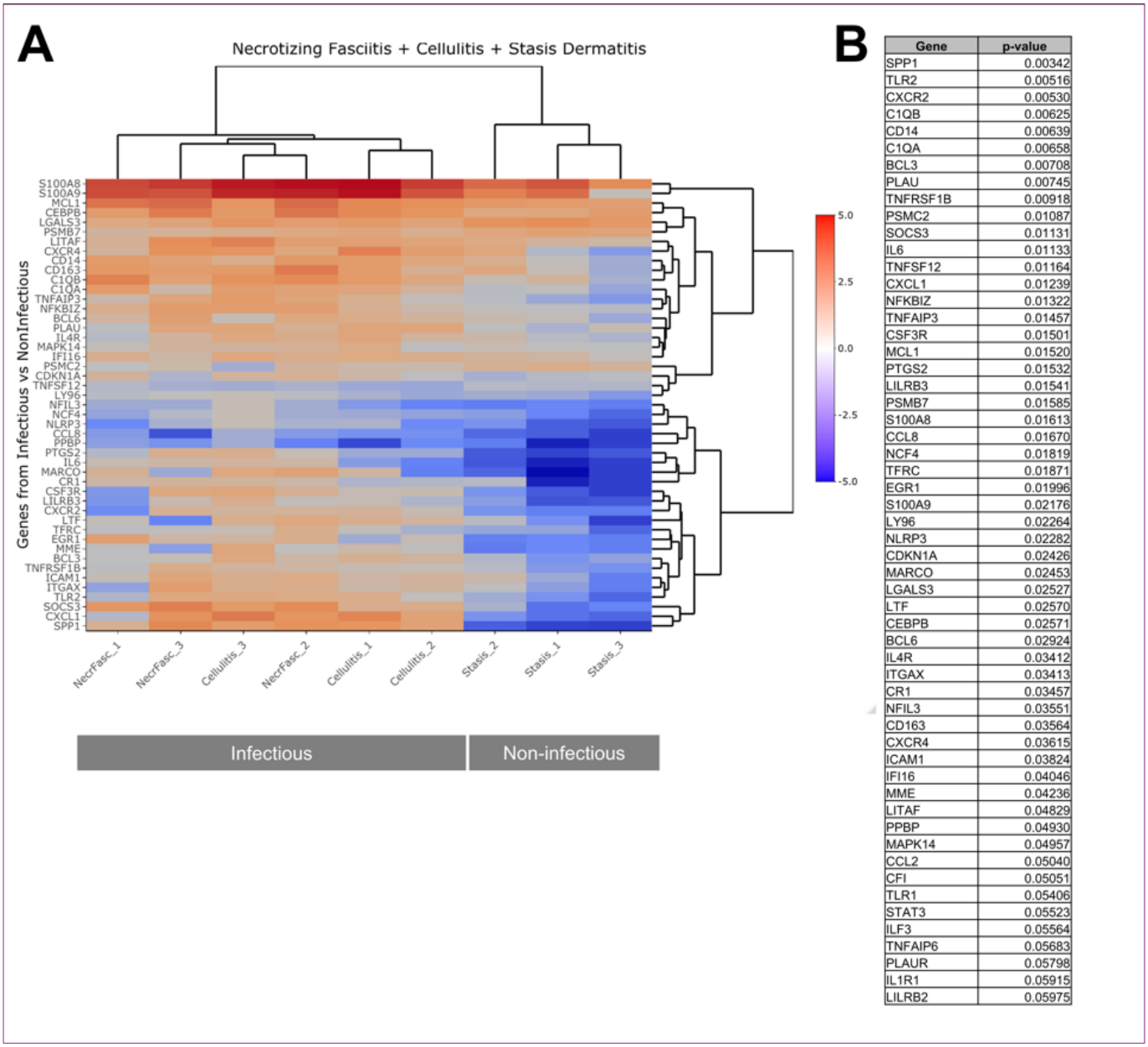


Figure 1. Flowchart of case selection for small-scale pilot study.



Gene	p-value
SPP1	0.00342
TLR2	0.00516
CXCR2	0.00530
C1QB	0.00625
CD14	0.00639
C1QA	0.00658
BCL3	0.00708
PLAU	0.00745
TNFRSF1B	0.00918
PSMC2	0.01087
SOCS3	0.01131
IL6	0.01133
TNFSF12	0.01164
CXCL1	0.01239
NFKBIZ	0.01322
TNFAIP3	0.01457
CSF3R	0.01501
MCL1	0.01520
PTGS2	0.01532
LILRB3	0.01541
PSMB7	0.01585
S100A8	0.01613
CCL8	0.01670
NCF4	0.01819
TFRC	0.01871
EGR1	0.01996
S100A9	0.02176
LY96	0.02264
NLRP3	0.02282
CDKN1A	0.02426
MARCO	0.02453
LGALS3	0.02527
LTF	0.02570
CEBPB	0.02571
BCL6	0.02924
IL4R	0.03412
ITGAX	0.03413
CR1	0.03457
NFIL3	0.03551
CD163	0.03564
CXCR4	0.03615
ICAM1	0.03824
IFI16	0.04046
MME	0.04236
LITAF	0.04829
PPBP	0.04930
MAPK14	0.04957
CCL2	0.05040
CFI	0.05051
TLR1	0.05406
STAT3	0.05523
ILF3	0.05564
TNFAIP6	0.05683
PLAUR	0.05798
IL1R1	0.05915
LILRB2	0.05975

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.[10] 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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