Peripheral Blood RNA Signatures Associated with Human Babesiosis, Quality of Life and Neurological Symptoms

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Introduction

- Over the past decade, the distribution of babesiosis in the US has changed. *Bm* transmission has expanded beyond the established endemic regions (i.e. the Northeast and upper Midwest states).
- There is also a marked increase in the number of immunosuppressed patients for whom treatment can be complicated and prolonged.
- Babesiosis is a potentially life-threatening disease caused by piroplasm parasites that infect RBCs yet host and parasite determinants of babesiosis in humans are completely unknown
- **Aim:** To identify parasite and host signatures of human babesiosis and correlate these with disease severity and long-term symptoms.

Materials and Methods

- **Enrollment:** The study takes advantage of two diverse clinical sites for enrollment of individuals with babesiosis; the Emergency Department at Stony Brook Medical Center and Southampton Hospital on Long Island since 2019.
- **Longitudinal cohort:** Blood samples are collected at initial infection (within 24h or before treatment) and subsequently at months 1, 6 and 12 post initial diagnosis.
- **Inclusion criteria:** any subject of 18 years-old or more who has acute babesiosis (microcopy positive blood smear for *Bm*, confirmed by PCR)
- **Exclusion criteria:** Individuals who present with babesiosis plus coinfection with other tick-borne diseases will be excluded from this study.
- **Clinical data:** All subjects complete a demographics survey and quality of life validated questionnaire, at initial infection and follow-ups visits.
- **Dual Total RNA-sequencing:** Total RNA from 5 mls of whole peripheral blood were isolated using the PAXgene Blood RNA Kit (IVD –FDA cleared system for molecular diagnostic testing) from individuals infected with *Bm* and healthy controls. Paired end RNA sequencing was performed using an Illumina NextSeq550. We will use STAR v2.4.2a, universal RNA-Seq aligner, to align results with a meta-genome comprised of human reference genomes.

Cohort Study (2019-ongoing) (n =64)	Total n (%)
Gender	47 (73.4)
Male	17 (26.6)
Female	
Age, mean in years (range)	61.2 (24-93)
Ethnicity	
Hispanic	26 (40.6)
Non-Hispanic	34 (53.1)
Unknown	4 (6.3)
Follow up visits	
Visit 1 (initial infection)	64* (100)
Visit 2 (1 month)	37 (68.5)
Visit 3 (6 months)	15 (27.7%)

* 10 subjects from 2018, only V1

- **Quality of Life (QOL) and Neurological symptoms (mean values)**
  - There was no significant difference ($p>0.05$) in scores of symptoms on the following variables (between time of presentation and 6 months):
  - Communication (V1=7.1; V2=5.8;V3=5.7)
  - Fatigue severity (V1=39.4;V2=33.7;V3=28)
  - SF36 General health (V1=271.4; V2=286; V3=255)

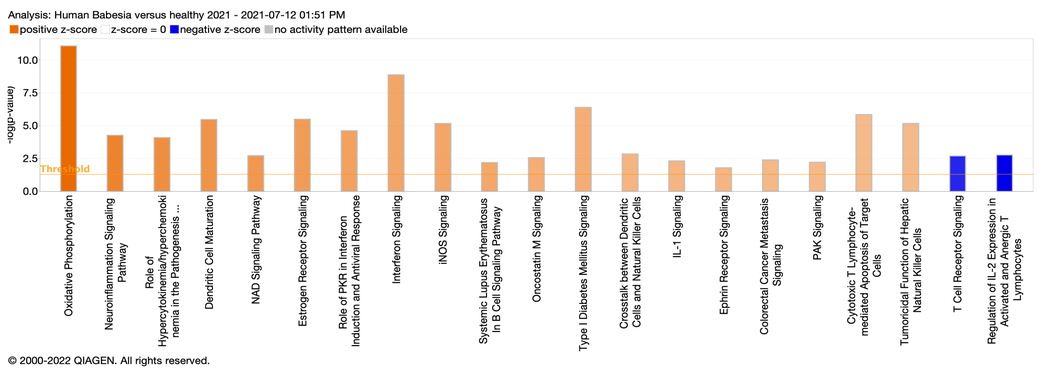
- Persistent symptoms assessed by VAS (mean values)

- VAS (visual analogue scale) was added in 2020.
- Out of 39 babesia subjects who completed VAS (n=39;V1=5.2), 60% continued having subjective symptoms at 6 months follow up (n=13; V2=5.5; V3=7.4) ($p>0.05$).

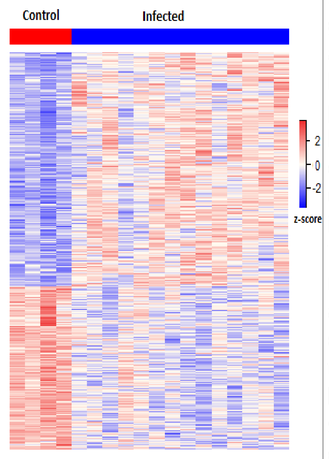
TRANSCRIPTOME

The peripheral blood transcriptome of a total of 17 patients (median age: 63 years, range 42-78; 24% female) who presented with babesiosis was distinctly different from uninfected individuals (n=9).

Transcriptome analysis showed Type I and Type II Interferon response. Other dominant transcriptional responses in the peripheral blood include those involved in activation of mononuclear cells, oxidative phosphorylation and regulation of homeostasis. iNOS pathways are also increased. Ingenuity Pathway Analysis for clinical chemistry and hematology also highlight changes associated with cardiotoxicity, hepatotoxicity, and nephrotoxicity as well as increased levels of red blood cells.



Results



IPA Heat Map Summary for 1947 differentially expressed genes (DEGs) (693down-, and 1254 up-regulated) based on FDR < 0.01 in patients who presented with babesiosis (14 patients) compared to healthy uninfected control individuals (4 individuals). The heatmap is based on results from DESeq2 analysis of RNA sequencing data and depicts log-transformed expression levels of individual genes, scaled using a z-score, for each gene (rows) across the 18 samples (columns). Blue and red row colors reflect low and high expression levels, respectively.

Conclusion

- This is a longitudinal cohort study on acute Babesia cases in Long Island, the results of this ongoing study is still in preparation.
- Based on these preliminary results, Babesiosis is associated with a marked alteration in the peripheral blood transcriptome of patients that may also provide insight into both the pathophysiology of the disease including severity and complications.
- Furthermore, even after successful treatment, babesiosis may result in long-term impacts on quality of life indicators.

***Results from this study are still preliminary.

References

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