Serum Pro-inflammatory Proteins Have Potential Utility as Biomarkers for NF-κB Targeting Approaches in DMD

Bista P[1]; Walter G[2]; Vandenborne K[3]; Lee B[2], Nichols A[1], Donovan J[1]

[1]Catabasis Pharmaceuticals, Cambridge USA; [2]University of Florida Physiology and Functional Genomics, Gainesville USA;
[3]University of Florida Health Physical Therapy, Gainesville USA

Introduction

- Duchenne Muscular Dystrophy (DMD) is a debilitating childhood-onset disease caused by dystrophin gene mutations.[1] Muscle function is progressively lost in DMD, with a total loss of ambulatory capacity around late adolescence.
- Activation of NF-κB in muscle occurs at an early age, regardless of mutation type, and is believed to be a central driver of inflammation, muscle degeneration and inhibition of muscle regeneration.[2]
- Glucocorticoids, the standard of care in DMD, can suppress inflammation and prolong ambulation, albeit with significant side-effects.[3]
- Magnetic Resonance Imaging (MRI) of leg muscles in young DMD boys can detect the progressive muscle inflammation and its delay with glucocorticoid therapy.[4]
- **Circulating blood markers that can correlate reliably with the status of NF-κB pathway activation in the muscle would allow efficient measurement of response to therapeutic interventions targeting NF-κB.**

Methods

- We performed an analysis of serum from 49 boys with DMD and 5 healthy controls (aged 4 to 18) across a panel of proteins.
- This sample set from the ImagingDMD cohort also included 3 boys that were steroid-naïve and longitudinal collections from 3 additional boys with DMD that transitioned into or out of glucocorticoid use (Table 1).
- Serum samples were analyzed using Meso Scale Discovery (MSD) electrochemiluminescent detection system.
- Fourteen serum proteins showed a significant correlation with age (Table 2).

Table 1

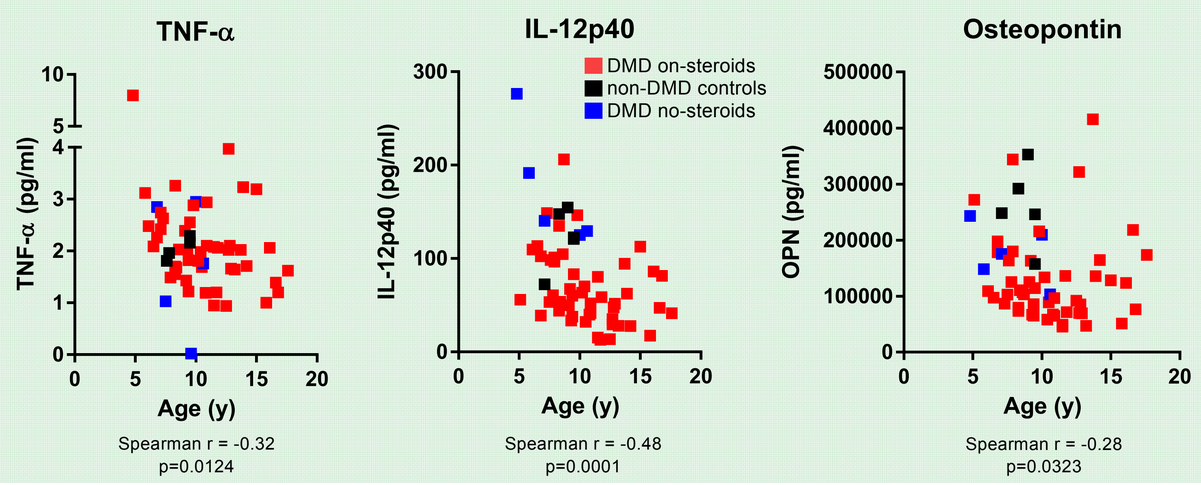
Sample Attribute	Age (Median)	Age (Range)
All (n=59 samples)	9.5	4.8-17.6
DMD on corticosteroids (n=48)	10.3	5.1-17.6
DMD not on corticosteroids (n=6)	8.4	4.8-10.6
Unaffected (n=5)	9.0	7.1-9.5

Table 2

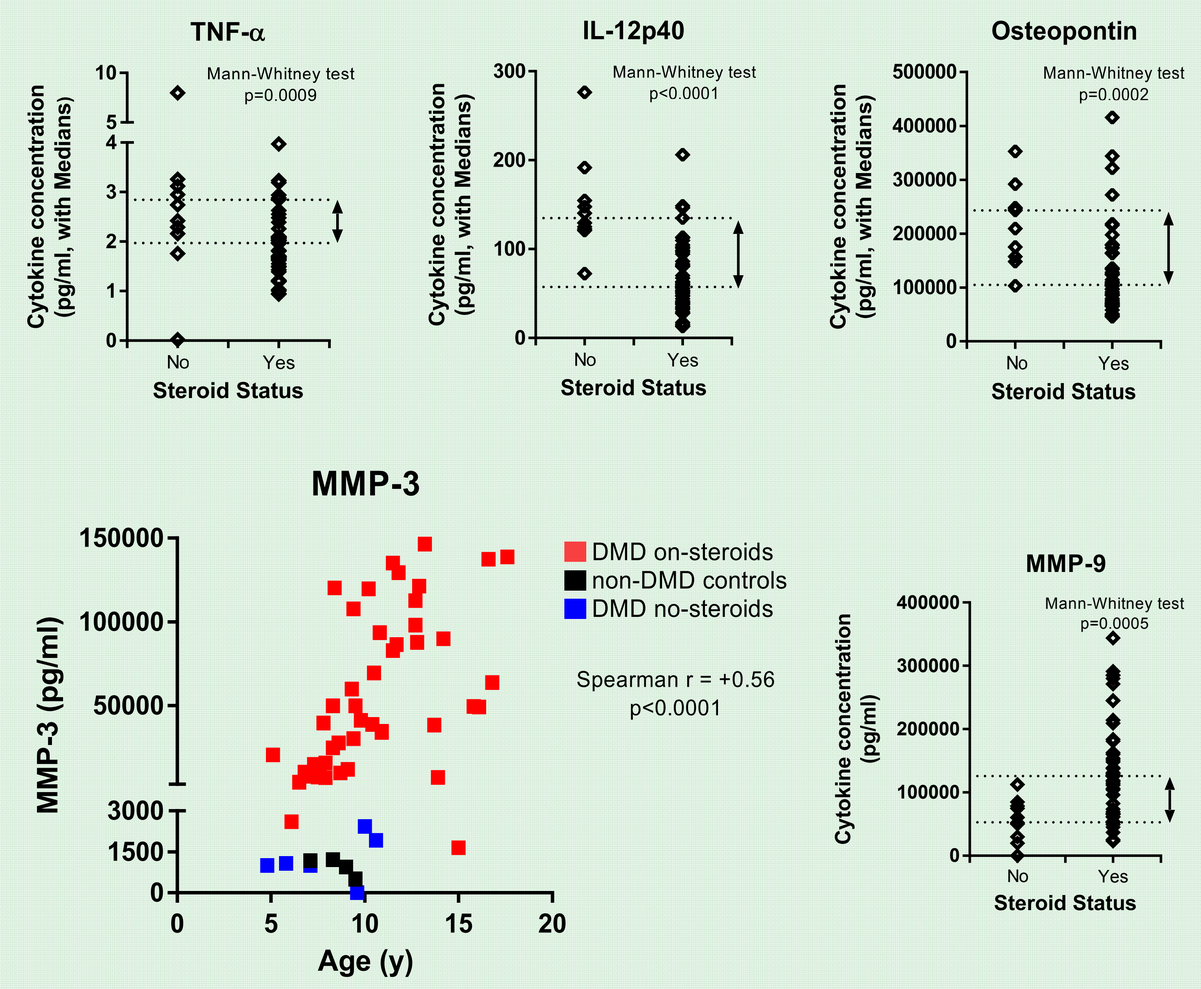
Analyte	Correlation with age	Spearman r (p-value)
MMP-3	Direct	+0.56 (<0.0001)
IL-12p40	Inverse	-0.48 (0.0001)
CRP	Inverse	-0.46 (0.0002)
GM-CSF	Inverse	-0.48 (0.0005)
TNF-β	Inverse	-0.43 (0.0010)
PIGF	Direct	+0.41 (0.0012)
IL-17	Inverse	-0.41 (0.0017)
ICAM-1	Inverse	-0.40 (0.0019)
IL-4	Direct	+0.49 (0.0023)
MMP-1	Direct	+0.34 (0.0084)
TNF-α	Inverse	-0.32 (0.0124)
MDC	Inverse	-0.30 (0.0208)
Osteopontin	Inverse	-0.28 (0.0323)
IFN-γ	Inverse	-0.26 (0.0448)

Results

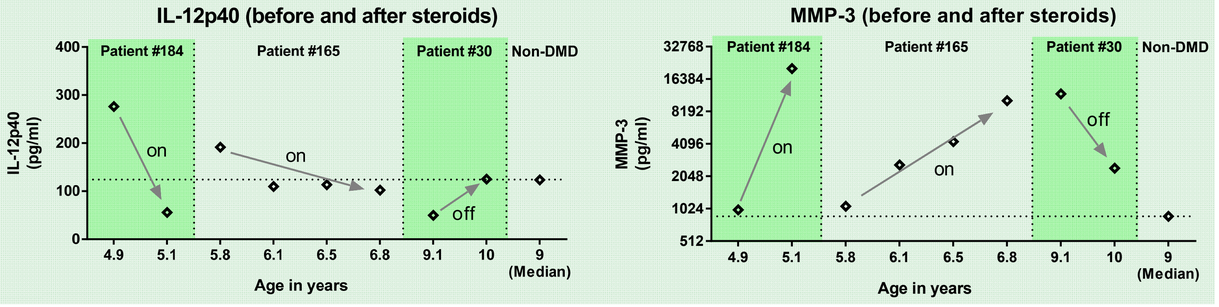
- Consistent with an early burden of muscle inflammation and NF-κB activity in DMD, levels of many serum proteins regulated by NF-κB such as TNF, IL-12, and Osteopontin (OPN) were highest in the youngest boys.



- Glucocorticoid treatment correlated with a decrease in the levels of these cytokines. However, steroid treatment correlated with elevated levels of MMP-9 and MMP-3.



- Changes in serum proteins were rapidly detected within 2-3 months of glucocorticoid use.



Conclusions

- In DMD serum, several NF-κB regulated cytokines showed an early peak and a progressive decline as the boys age.
- These cytokines decreased with glucocorticoid treatment, suggesting a glucocorticoid effect on the regulation of NF-κB mediated inflammation.
- On the other hand, MMP-3 and MMP-9 levels in serum were increased with glucocorticoid treatment, suggesting additional modes of glucocorticoid action.
- The rapidly detectable changes in NF-κB driven proteins in serum suggest potential utility for these circulating proteins as biomarkers in therapeutic approaches targeting NF-κB in DMD.

Acknowledgments

We express our deepest gratitude to the boys with DMD and their families who continually share parts of their lives with us.

References

1. Kunkel, LM, et. al., *Nature* 1986; 322:73-77
2. Chen, YW, et. al., *Neurology* 2005; 65:826-834
3. Fenichel, et. al., *Neurology* 1991; 14:1874-
4. Arpan, I, et. al., *Neurology* 2014; 83:974-980, and Willcocks, RJ, et. al., *Neuromuscul Disord* 2015; 24:393-401