Stem Cell Factor Associated with Critical Limb Ischemia in Patients with Peripheral Artery Disease

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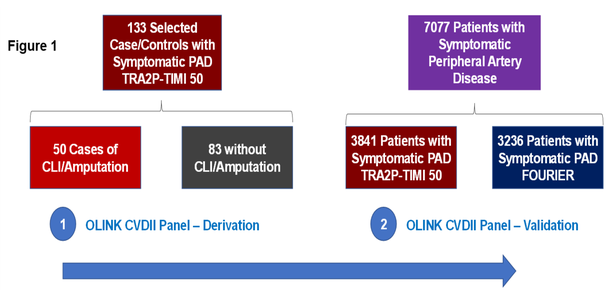
BACKGROUND

- Approximately 12 million people in the United Sates and 230 million globally have peripheral artery disease (PAD).
- Major adverse limb outcomes, and in particular amputation, remains a feared outcome which is associated with disability and increased risk of mortality
- Patients with PAD diabetes mellitus (DM) are at further heightened risk of amputation, with often multifactorial etiologies, predominantly infection in the setting of ischemia and neuropathy
- Prevention of amputation is complex due to its complex pathobiology and novel biomarkers hold promise both in terms of risk stratification as well as elucidating novel pathways which may enable development of therapeutic interventions

METHODS

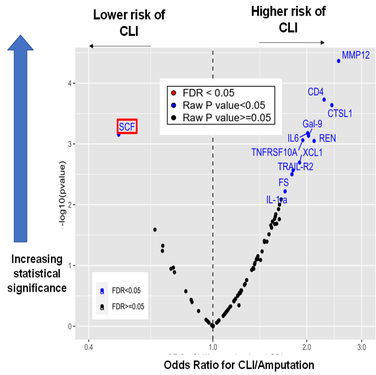
- Two large clinical trials which enrolled patients with symptomatic lower extremity PAD and had biosamples were utilized: TRA2P-TIMI 50 NCT00527943 and FOURIER NCT01764633
- Major adverse limb events including amputation, critical limb threatening ischemia (CLTI) , acute limb ischemia, and revascularization were adjudicated by two blinded vascular specialists.
- A total of 50 patients with peripheral artery disease who suffered CLTI or amputation and 83 who did not were selected for derivation (Figure 1 left). Samples underwent testing for proteomic exploration with the Olink CVDII panel at the BWH / TIMI biomarker core lab.

Figure 1



RESULTS

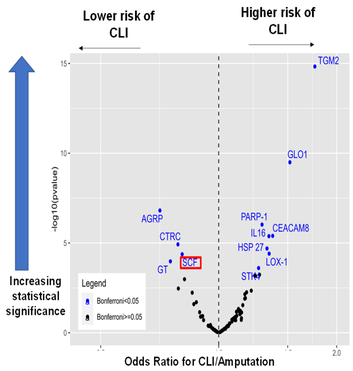
Figure 2



Assay	# of Cases	# of Controls	OR per 1 SD	P-Value	Bonferroni	FDR
MMP12	50	83	2.53	<0.001	0.004	0.004
CD4	50	83	2.27	<0.001	0.017	0.0071
CTSL1	50	83	2.41	<0.001	0.021	0.0071
IL6	50	83	2.01	<0.001	0.061	0.010
SCF	50	83	0.50	<0.001	0.065	0.010
Gal-9	50	83	2.03	<0.001	0.068	0.010
TNFRSF10A	50	83	1.94	<0.001	0.08	0.010
REN	50	83	2.11	<0.001	0.082	0.010
XCL1	50	83	1.90	<0.001	0.19	0.020
TRAIL-R2	50	83	1.81	<0.001	0.25	0.025
FS	50	83	1.79	<0.001	0.29	0.026
IL-1ra	50	83	1.70	<0.001	0.55	0.046

- Candidate markers were evaluated for their association with the outcome of CLTI/Amputation. A total of 12 markers were significantly associated with CLTI/Amputation, including 11 with a positive association and 1 with a negative association (Figure 2).

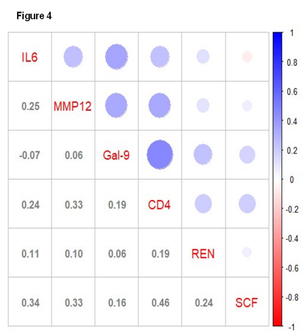
Figure 3



Assay	# of Cases	# of Controls	OR per 1 SD	P-Value	Bonferroni	FDR
SCF	242	6766	0.81	<0.001	0.000465	0.00047
REN	242	6767	1.27	<0.001	0.006427	0.0032
Gal-9	242	6767	1.21	0.0045	0.049786	0.017
TNFRSF10A	242	6767	1.17	0.013	0.14	0.034
CD4	242	6766	0.86	0.025	0.28	0.056
MMP12	242	6767	1.13	0.064	0.71	0.11
IL6	242	6767	1.11	0.067	0.74	0.11
CTSL1	242	6767	1.05	0.45	1	0.55
XCL1	242	6767	1.01	0.87	1	0.87
TRAIL-R2	242	6767	1.08	0.19	1	0.26
FS	242	6767	1.01	0.84	1	0.87

- A validation step utilized the entire 7,077 patients in the combined data sets who were included with symptomatic PAD (Figure 1 right). A total of 14 markers were significantly associated with CLTI/Amputation, 3 of which were identified in the derivation step (Figure 3, panel right).

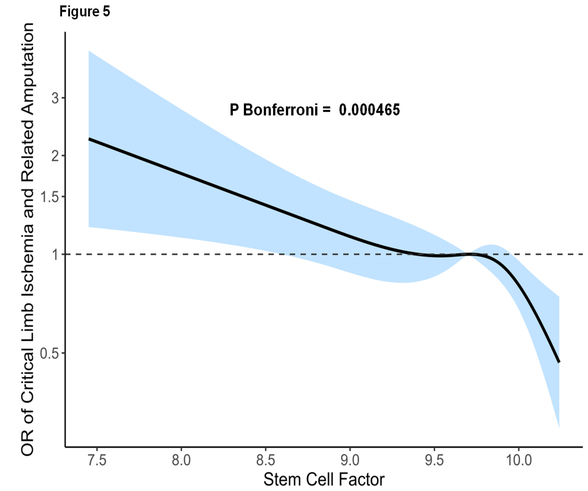
Figure 4



- The strongest statistical relationship was a negative association between Stem Cell Factor (SCF) and the risk of CLTI/Amputation.
- SCF was the only marker that was inversely associated with this outcome
- The correlation between the associated markers is shown in Figure 4.

RESULTS

Figure 5



- Stem Cell Factor (SCF) is also known as KIT-Ligand (KL) and is a cytokine that binds to the c-KIT (CD117) receptor
- SCF is expressed by endothelial cells and is associated with hematopoiesis and vasculogenesis
- A cubic spline of SCF concentration and CLTI/Amputation showed a continuous inverse relationship with risk (Figure 5)

Table 1

Characteristics	SCF < median (N=3504)	SCF >= median (N=3504)	P-value
Age, Median (Q1-Q3)	62.0 (57.0 - 68.0)	67.0 (61.0 - 73.0)	<0.001
Female Sex	24.4%	32.0%	<0.001
White Race	91.6%	88.8%	<0.001
Hypertension	81.9%	85.2%	<0.001
Hyperlipidemia	93.1%	93.6%	0.72
Current Smoking	41.4%	23.7%	<0.001
Diabetes Mellitus	38.9%	41.4%	0.11
eGFR < 60	47.0%	53.2%	<0.001
Prior Revasc.	52.6%	50.7%	0.29
Heart Failure	15.3%	18.1%	0.007
Prior Amputation	4.6%	3.9%	0.43
Statin Therapy	89.5%	92.4%	<0.001
Aspirin Therapy	85.0%	83.2%	0.11
Insulin	13.8%	16.4%	0.011

- Baseline characteristics of patients with PAD in the combined data sets stratified by SCF at the median are shown in Table 1
- Patients with increased SCF (associated with lower risk of CLTI/Amputation) were older, more likely to be female sex, less likely to be of white race, less likely to be smoking. Those with higher SCF were more also likely to have hypertension, an estimated glomerular filtration rate (eGFR) less than 60, have a history of heart failure, and to be treated with statins and insulin (Table 1)

RESULTS

Figure 6

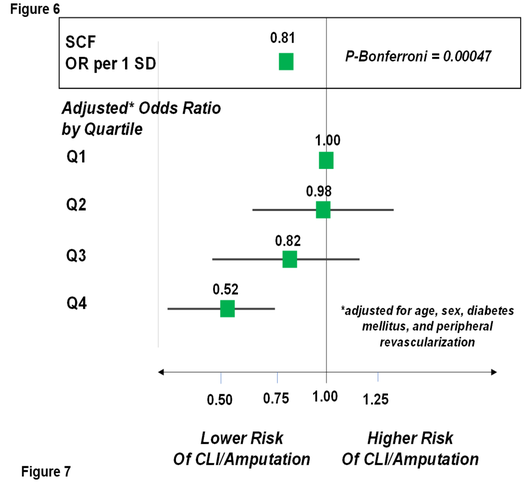
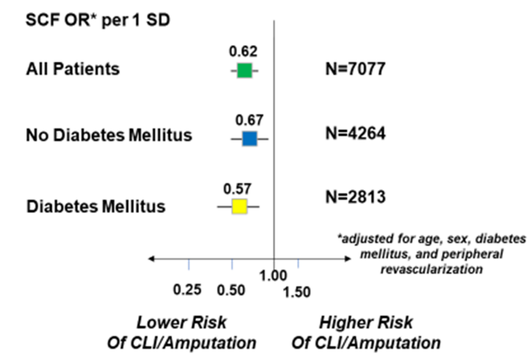


Figure 7



- Examined by quartiles and adjusting for baseline differences (Figure 6), a graded inverse association between SCF and CLI/AMP was observed (Q4 vs Q1 Adjusted OR 0.52, 95% CI: 0.34, 0.77; p=0.002)
- The relationship was consistent regardless of comorbid diabetes (Figure 7) There was no treatment interaction between SCF and randomized treatment with vorapaxar (p-interaction 0.55) or evolocumab (p-interaction 0.96)

LIMITATIONS

- Power for the discovery step was limited by the number of cases and controls and as a result, some associated markers may have been selected using the FDR threshold.
- Validation in additional data sets is required

SUMMARY & CONCLUSION

- A proteomic approach to identifying novel biomarkers associated with CLI/AMP revealed a strong inverse risk relationship with SCF levels.
- These data suggest that SCF and its effects on vasculogenesis warrant further investigation as a pathway to prevent and treat CLI/AMP in patients with PAD.

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