Intra-articular VEGF and MMP-1 are the Primary Drivers of Worse Baseline KOOS Symptoms and Quality of Life Subscores at Time of Knee Chondroplasty

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Introduction: Biomarkers are a topic of interest in orthopaedics in the setting of osteoarthritis and patients undergoing knee arthroscopy for any reason. However, in patients undergoing knee arthroscopy for chondral defects, the influence of cytokines on patient pain and function is not fully understood. The purpose of this study is to investigate the concentrations of synovial inflammatory cytokines in patients undergoing arthroscopic chondroplasty for chondral defects in the knee and correlate those cytokine levels with baseline patient reported outcome measures (PRO's) and defect characteristics. We hypothesized that the synovial cytokine environment will correlate with patient symptoms more so than baseline defect characteristics.

Methods: Sixty patients 18-50 years old undergoing arthroscopic chondroplasty for knee cartilage defects were enrolled. Patients were assigned preoperative Knee injury and Osteoarthritis Outcome Score (KOOS) and International Knee International Knee Documentation Committee (IKDC) Subjective Knee Forms. Preoperative magnetic resonance images were used to calculate AMADEUS (Area Measurement And Depth Underlying Structure) scores.[1] All patients received a successful synovial fluid aspiration just prior to initiation the arthroscopic procedure. The number of defects, total defect area, and ICRS grades were recorded based on intraoperative assessment. Patients with concomitant procedures beyond partial meniscectomy were excluded.
Multiplex ELISA analyzed aspirations for: PDGF-BB, CCL-5/RANTES, MMP-3, MMP-1, EGF, VEGF, IL-1a, FGF-2, CCL-2, BMP-2, and aggrecan. Univariate correlation testing was used to assess significance between cartilage defect characteristics, PROs, and cytokine concentrations **(Figure 1).** The Akaike Information Criterion (AIC) was utilized to select the best-fit multivariate regression model using the 4 most significant independent variables for each cytokine **(Figure 2)**. AIC best-fit modeling was repeated for each PRO that had at least two significant cytokine associations on univariate testing. **(Figure 3).** Significance was set at $P<0.05$.

Results: MMP-1 had a positive correlation with number of defects treated (P=0.016) and negative correlation with KOOS quality of life (QOL) subscores (P=0.035; R^2 =0.173)**.** VEGF was positively correlated with defects treated (P=0.005) and negatively correlated with KOOS Symptoms scores (P=0.035; R^2 =0.225). The treatment of multiple defects was an independent predictor of elevated IL-1a (P=0.002, R^2 =0.202). CCL-2 was positively correlated with multiple defects (P=0.012) and negatively with KOOS QOL (P=0.016, R^2 =0.173). Female sex was correlated with higher concentrations of MMP-3 (P=0.007, R^2 =0.144), FGF (P=0.012, R^2 =0.178), and BMP-2 (P=0.008; R^2 = 0.169). BMP-2 was negatively correlated with KOOS Symptom (P=0.019). The primary driver of preoperative KOOS Symptoms scores on multivariate analysis was VEGF (P=0.023, R^2 =0.120), influencing patient symptoms beyond defect characteristics. Similarly, KOOS QOL was independently correlated with MMP-1 concentration (P= 0.045, R^2 =0.079), more so than ICRS grade (**Figure 3**).

Discussion: Multivariate regression analysis revealed that elevated MMP-1 was the primary driver of worse preoperative KOOS QOL scores, more so than defect characteristics such as the number of lesions treated. Similarly, worse preoperative KOOS Symptoms scores were more strongly correlated with elevated VEGF concentrations rather than defect ICRS grades.

Clinical Relevance: Our study demonstrated that synovial fluid cytokines appear to be the primary drivers of patients' subjective assessments of their pain and function preoperatively.

	Statistical Test Used		PDGF	CCL-5	MMP-3	MMP-1	EGF	VEGF	IL-1a	FGF	CCL-2	BMP-2	Aggrecan
Independent Variables	*Spearman Correlation*	IKDC	p=0.616 rho= – 0.073	p=0.749 rho= – 0.047	p=0.781 rho= – 0.041	p=0.091 rho= – 0.244	p=0.616 rho= – 0.074	p=0.828 rho= – 0.032	p=0.670 rho= – 0.062	p=0.380 rho= – 0.128	p=0.938 rho= – 0.011	p=0.824 rho= – 0.033	p=0.575 rho= – 0.084
	Spearman Correlation	KOOS Pain	p=0.307 rho= 0.142	p=0.323 rho= 0.137	p=0.886 rho= – 0.020	p=0.175 rho= – 0.187	p=0.295 rho= 0.145	p=0.063 rho= – 0.254	p=0.411 rho= – 0.114	p=0.976 rho= 0.004	p=0.143 rho= – 0.202	p=0.712 rho= 0.051	p=0.756 rho= – 0.043
	Spearman Correlation	KOOS Symptoms	p=0.739 rho= 0.046	p=0.592 rho= 0.075	p=0.493 rho= 0.095	***p=0.026 rho= – 0.302**	p=0.885 rho= 0.020	***p=0.002 rho= – 0.406**	p=0.293 rho= – 0.146	p=0.225 rho= 0.168	p=0.349 rho= – 0.130	p=0.295 rho= 0.145	p=0.555 rho= – 0.082
	Spearman Correlation	KOOS ADL	p=0.332 rho= 0.135	p=0.291 rho= 0.146	p=0.539 rho= – 0.086	p=0.095 rho= – 0.230	p=0.325 rho= 0.137	p=0.070 rho= – 0.249	p=0.356 rho= – 0.128	p=0.706 rho= – 0.053	***p=0.049 rho= – 0.269**	p=0.960 rho= – 0.007	p=0.472 rho= – 0.100
	Spearman Correlation	KOOS Sport	p=0.628 rho= – 0.067	p=0.568 rho= – 0.080	p=0.817 rho= 0.032	p=0.057 rho= – 0.261	p=0.430 rho= – 0.110	p=0.100 rho= – 0.226	p=0.442 rho= – 0.107	p=0.974 rho= 0.005	p=0.476 rho= – 0.099	p=0.851 rho= – 0.026	p=0.906 rho= – 0.016
	Spearman Correlation	KOOS QOL	p=0.252 rho= – 0.159	p=0.256 rho= – 0.157	p=0.577 rho= – 0.078	***p=0.025 rho= – 0.306**	p=0.208 rho= – 0.174	p=0.260 rho= – 0.156	***p=0.017 rho= – 0.324**	p=0.393 rho= – 0.118	p=0.121 rho= – 0.214	p=0.320 rho= – 0.138	***p=0.032 rho= – 0.293**
	Spearman Correlation	KOOS Jr	p=0.518 rho= 0.089	p=0.583 rho= 0.076	p=0.851 rho= – 0.026	p=0.121 rho= – 0.211	p=0.739 rho= 0.046	***p=0.049 rho= – 0.266**	p=-0.324 rho= – 0.136	p=0.729 rho= 0.048	p=0.122 rho= – 0.211	p=0.809 rho= 0.033	p=0.656 rho= – 0.061
	Spearman Correlation	Total Defect Area	p=0.362 rho= – 0.125	p=0.216 rho= – 0.170	p=0.340 rho= – 0.123	***p=0.012 rho= 0.340**	p=0.593 rho= – 0.074	***p=0.009 rho= 0.352**	p=0.051 rho= 0.267	p=0.153 rho= – 0.197	p=0.205 rho= 0.175	p=0.735 rho= – 0.047	p=0.469 rho= 0.101
	Spearman Correlation	AMADEUS Score	p=0.503 rho= 0.091	p=0.174 rho= 0.184	p=0.531 rho= 0.085	p=0.273 rho= – 0.149	***p=0.042 rho= 0.273**	p=0.532 rho= – 0.085	p=0.997 rho= 0.000	p=0.220 rho= 0.167	p=0.843 rho= – 0.027	p=0.301 rho= 0.141	p=0.634 rho= – 0.065
	Spearman Correlation	ICRS Grade	p=0.091 rho= – 0.232	p=0.106 rho= – 0.222	p=0.768 rho= – 0.041	p=0.426 rho= 0.111	p=0.152 rho= – 0.216	p=0.600 rho= 0.073	p=0.308 rho= 0.141	p=0.148 rho= – 0.106	p=0.453 rho= 0.104	p=0.518 rho= – 0.091	p=0.251 rho= – 0.159
	Kruskal-Wallis	Number of Defects Treated (1/2/3/4)	p=0.772	p=0.999	p=0.856	***p=0.035**	p=0.719	***p=0.002**	p=0.058	p=0.725	p=0.065	p=0.226	p=0.282
	Mann-Whitney U	Number of Defects Treated (Single/Multiple)	p=0.788	p=0.957	p=0.763	p=0.076	p=0.167	***p=0.004**	***p=0.037**	p=0.825	***p=0.015**	p=0.282	p=0.213
	Spearman Correlation	Age	p=0.966 rho= 0.006	p=0.309 rho= – 0.134	p=0.791 rho= – 0.035	p=0.116 rho= 0.205	p=0.593 rho= 0.070	p=0.077 rho=0.230	p=0.069 rho=0.237	p=0.638 rho= – 0.062	p=0.715 rho= 0.048	p=0.513 rho= – 0.086	p=0.180 rho= 0.175
	Mann-Whitney U	Sex (Female/Male)	p=0.677	p=0.298	***p=0.014**	p=0.647	p=0.494	p=0.712	p=0.443	***p=0.014**	p=0.791	***p=0.040**	p=0.443
	Mann-Whitney U	Concomitant Procedure (Yes/No)	p=0.888	p=0.767	p=0.092	p=0.747	p=0.830	p=0.930	p=0.148	***p=0.020**	p=0.577	p=0.587	p=0.239

Figure 1. Results from univariate correlation testing between PRO's, defect characteristics, demographic info, and intraoperative synovial fluid aspiration. A rho >0 represents a positive correlation, while rho <0 represents negative correlation.
* Signifies statistical significance. Red highlight = significant negative correlation. Green highlight = significant positive correlation. Yellow highlight = significant difference between groups, rho not applicable.

Patient Reported Outcome Score	KOOS Symptoms	KOOS QOL
Independent Variables Analyzed	1. Total Defect Area 2. VEGF 3. Number of Defects 4. MMP-1	1. IL-1 2. MMP-1 3. Aggrecan 4. ICRS Grade
AIC Best-Fit Model (p-value)	**VEGF (0.023)*** Total Area (0.186)	**MMP-1 (0.045)*** ICRS Grade (0.151)
Adjusted R2	0.120	0.079

Figure 3. Multivariate regression modeling based on AIC best-fit models for the PRO scores with at least two significant correlations to synovial cytokine concentrations based on univariate analyses. Variables found to be independently correlated with PRO scores on multivariate regression analysis are marked in bold with *.

Cytokine	PDGF	CCL-5	MMP-3	MMP-1	EGF	VEGF	IL-1a	FGF	CCL-2	BMP-2	Aggrecan
Independent Variables Analyzed	1. KOOS Pain 2. KOOS ADL 3. KOOS QOL 4. ICRS Grade	1. ICRS Grade 2. AMADEUS 3. KOOS QOL 4. KOOS ADL	1. Sex 2. Concomitant 3. Total Defect Area 4. KOOS Symptoms	1. Number of defects 2. KOOS QOL 3. KOOS Symptom 4. KOOS Sport	1. AMADEUS 2. KOOS QOL 3. KOOS Pain 4. KOOS ADL	1. Number of defects 2. KOOS Symptoms 3. Defect Area 4. KOOS Jr	1. KOOS QOL 2. Number of defects 3. Concomitant procedure 4. ICRS Grade	1. Sex 2. Concomitant procedure 3. ICRS Grade 4. Defect Area	1. Number of defects 2. KOOS QOL 3. KOOS Jr 4. KOOS Pain	1. Sex 2. Number of defects 3. KOOS Symptoms 4. AMADEUS	1. KOOS QOL 2. Age 3. Number of defects 4. ICRS Grade
AIC Best-Fit Model (p-value)	KOOS ADL (0.063) ICRS Grade (0.9612)	ICRS Grade (0.563) AMADEUS (0.054) KOOS QOL (0.267)	**Sex (0.007)*** Defect Area (0.825) KOOS Symptom (0.355)	**Number of defects (0.012)*** **KOOS QOL (0.016)***	AMADEUS (0.056) KOOS QOL (0.141)	**Number of defects (0.005)*** **KOOS Symptoms (0.035)*** Defect Area (0.178)	**Number of defects (0.035)*** KOOS QOL (0.057) ICRS Grade (0.836)	**Sex (0.013)*** Defect area (0.246) Concomitant procedure (0.120) ICRS Grade (0.171)	**Number of defects (0.012)*** **KOOS QOL (0.016)***	**Sex (0.008)*** **KOOS Symptoms (0.019)*** AMADEUS (0.479)	KOOS QOL (0.085) ICRS Grade (0.069)
Adjusted R2	0.032	0.044	0.114	0.173	0.06808	0.225	0.095	0.178	0.173	0.169	0.068

Figure 2. AIC best-fit modeling determined the greatest amount of variation using the fewest possible independent variables for each cytokine. Variables found to be independently significant by subsequent multivariate regression analysis are marked in bold with *.