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Article

Pre-Operative Adiposity and Synovial Fluid Inflammatory Biomarkers Provide a Predictive Model for Post-Operative Outcomes Following Total Joint Replacement Surgery in Osteoarthritis Patients

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Abstract: A proportion of osteoarthritis (OA) patients are unsatisfied with post-operative outcomes following total joint replacement surgery (TJR), with insufficient pain relief or poor functional improvement. Predicting those will have poor outcomes would be beneficial for patients and clinicians. The aim of this study was to determine the relationship between baseline anthropometric data and the concentration of pre-operative serum and peri-operative synovial fluid (SF) cytokines with 7-month post-operative outcomes in a cohort of knee and hip OA patients. 160 OA patients were recruited who were scheduled for TJR. The concentration of 24 cytokines were measured in blood and SF by multiplex assay. EQ5Dindex health status was assessed pre-operatively and at 7-months post-operatively. 13% of patients were identified as non-responders based on EQ5Dindex. Compared to responders, non-responders were of higher BMI, had greater waist and hip circumference and had higher levels of SF leptin but lower levels of SF Resistin ($p<0.05$). Linear regression analysis found a significant relationship between pre-operative body weight and post-operative response (ΔEQ5Dindex; $r=0.222$, $p=0.049$). The combination of body weight with SF amphiregulin and SF IL-6 provided an improved predictive model of post-operative response ($r=0.470$, $p=0.035$).

Keywords: osteoarthritis; post-operative outcomes; pain; health status; cytokines; obesity; inflammation

1. Introduction

With an aging population and increasing incidence of obesity the number of total joint replacements performed each year is increasing and is projected to continue to increase [1]. The main indication for the procedure is osteoarthritis (OA). In 2018, more than 95,000 hip and 100,000 knee total joint replacements surgeries were performed in England, Wales and Northern Ireland. Unfortunately, based on the quality of life patient questionnaires (EQ-5D and Oxford Knee Score), a proportion of patients report dissatisfaction following joint replacement surgery [2]. Approximately 10% of hip OA patients and 20% of knee OA patients report having a poor clinical outcome, mainly characterised by a lack of pain relief or poor improvement in function [3].

In attempting to identify prognostic biomarkers of poor post-operative outcome several studies have examined the association between serum and synovial fluid concentrations of pro-inflammatory cytokines and pain in OA patients [4, 5] since it is known that such inflammatory factors are capable of promote the sensitisation of nociceptors [6, 7] . Indeed, it has previously been reported that patient reported pain is associated with distinct patterns of synovitis [8]. Further, that specific sites of patient reported pain in knee OA patients are associated with greater synovitis , with synovial tissue that exhibited distinct fibroblast subsets that promoted neuronal growth and survival [9]. Furthermore, high concentrations of pre-operative synovial fluid TNFα and IL-6 were associated with increased post-operative pain at two year follow up [10]. In a separate study, a significant correlation was reported between peak CRP levels and the level of pain and stiffness at 2 days post-operatively [11].

However, to date no study has performed multiplexing profiling of both peri-operative synovial fluid and pre-operative serum cytokines and analysed their associations with post-operative joint replacement outcomes in patients with knee and hip OA. Therefore, the aim of this study was to investigate both peri-operative synovial fluid and pre-operative cytokines as potential predictive biomarkers of joint replacement outcome in a cohort of patients with osteoarthritis.

2. Materials and Methods

2.1. Patients

Following ethical approval (UK National Research Ethics Committee 14/ES/1044), 160 OA patients were recruited to the study who were scheduled to undergo total hip replacement surgery (n=97) or total knee replacement surgery (n=63) at either the Royal Orthopaedic Hospital, Birmingham, UK or Russells Hall Hospital, Dudley, UK. Pre-operatively, anthropometric data (including BMI, fat%, waist: hip ratio) were recorded. Patients completed EQ5D [12] pre-operatively and at 7 months post-operatively. Following completion of questionnaires, pre and post-operative data were available for EQ5Dindex and the five components of EQ5D, namely "mobility", "usual activities", "self-care", "pain/discomfort" and "anxiety/depression". Pre-operatively, blood samples were collected and peri-operatively synovial fluids were aspirated from the joint.

2.2. Quantification of Serum and Synovial Fluid Cytokines by Multiplex Bead Assay

To determine cytokine and chemokine concentrations in serum and synovial fluids, multiplex technology (Luminex® Screening Assay, R&D Systems) was performed. In the case of synovial fluid, samples were treated with 2mg/mL hyaluronidase as previously described [13]. Multi-plex analysis was performed according to the manufacturer's instructions. In brief, 50 µl of a 1x antibody magnetic bead stock (Adiponectin, Serpin E1, Aggrecan, Amphiregulin, CCL11, CCL2, CCl3, CCL20, Chemerin, CXCL10, Dkk1, Galectin-1, gp130, IL1β, IL10, IL15, IL7, Visfatin, TNF-α, Galectin-3, Galectin-3BP, Lipocalin-2, CCL-4, FABP4, LIF, Leptin, IL6, Resistin, and MMP-1, -2, -3, -7, -8, -9, -10, -12, -13) was added to each well of a 96 well plate. 50 µl of standard solution or serum or hyaluronidase-treated synovial fluid was then added to relevant wells and incubated for 2 hr. Post incubation, the plate was washed 3x with wash buffer and 50 µl of a biotinylated antibody added to all wells. Following a 1 hr incubation, the plate was washed 3x and 50 µl of diluted streptavidin-PE added to all wells. After a further 30 min incubation in the dark, the plate was washed as before and cytokine concentrations analysed using a Luminex® 200™ instrument (Luminex® Corporation, Austin, Texas, USA).

2.3. Statistical Analysis

Data distribution was assessed using Kolmogorov- Smirnov test and was found to not be normally distributed. Mann-Whitney tests were performed to determine significance. Data are presented as Median with Interquartile Range (IQR). Univariate and multivariate linear regression analysis was performed using SPSS v24 software.

3. Results

3.1. Identification of Patients with Poor Post-Operative Outcomes and Analysis of EQ5D Index Components

Based on pre-operative and post-operative EQ-5D index, 87% (n=139) of patients had positive post-operative outcomes and were classified as 'responders'. The remaining 13% (n=21) of patients, who either had a negative outcome or no improvement, were classified as "non-responders" (Figure 1a, b and Table 1). When comparing outcome between hip and knee joint replacement separately, 89% (n=86) of patients had positive response following hip replacement compared to 84% (n=53) who had positive response following knee replacement (Figure 1a, b and Table 1).

For the pain/discomfort EQ5D component, 72% (n=123) of patients improved following the surgery (Table 1). When comparing outcome between hip and knee joint replacement separately, 77% (n=81) of patients had positive pain/discomfort improvement following hip replacement compared to 63% (n=42) who had positive pain/discomfort improvement following knee replacement (Table 1).

Table 1. The comparison of post-operative outcomes between knee and hip joint replacement surgery. Data are represented as percentage and number of patients (% (n)) with positive response, negative response and no change between pre-operative scores and post-operative scores.

	All Joints	Hips	Knees
EQ5Di			
Responders	87% (139)	89% (86)	84% (53)
Non-responders	13% (21)	11% (11)	16% (10)
Pain/discomfort			
Responders	72% (123)	77% (81)	63% (42)
Non-responders	28% (49)	23% (24)	37% (25)

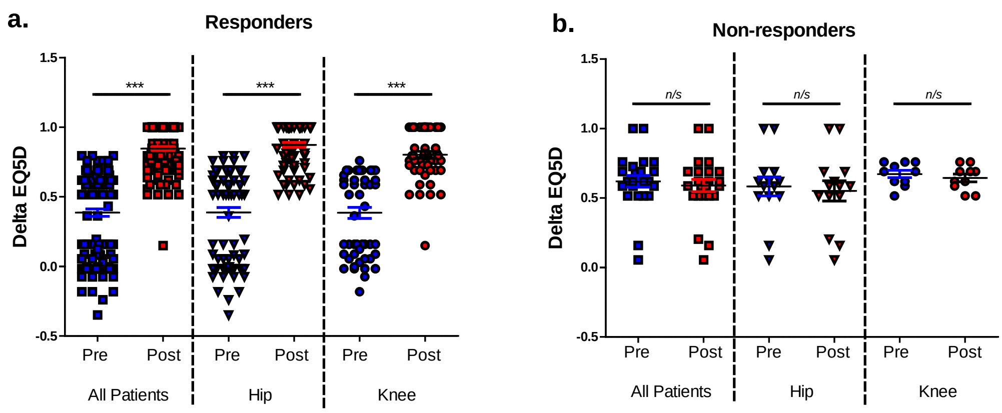

Figure 1. Responders and non-responders defined by change in EQ5D index between pre-operative and 7-month post-operative. (**a**) responder patients, (**b**) non-responder patients. ***=significantly different between pre and post joint replacement surgery, p<0.001.

Analysis of individual EQ5D components showed that responders improved in each of the EQ5D components, namely mobility, self-care, usual activities, pain/discomfort and anxiety/depression. The greatest improvements were in the mobility EQ5D component with 100% of hip OA and 100% of knee OA responder patients reporting no problem with their post-operative mobility (Table 2). Notably, the EQ5D pain component showed the least improvement post-operatively, with 15% of hip OA responders and 43% of knee OA responders still reporting "Level 2, some problem". In contrast, those patients identified as non-responders saw no improvement in any of the EQ5D components (Table 2).

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Table 2. Pre and post-operative EQ5D index. EQ-5D index was assessed across 5 different components-mobility, self-care, usual activities, pain/discomfort and anxiety/depression, scored across 3 levels, namely L1 (no problem, L2 (some problem) and L3 (severe problem). Data is represented as % and number of patients (% (n)) for each component and each level of EQ5D.

	Mobility		Self -Care		Usual Activities		Pain/Discomfort		Anxiety/Depression	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Non-responders										
All										
L1	19%(14)	18%(13)	80%(91)	75%(86)	17%(12)	13%(9)	4%(2)	4%(2)	80%(95)	77%(92)
L2	81%(60)	82%(61)	20%(23)	24%(27)	80%(57)	83%(59)	89%(41)	89%(41)	19%(23)	22%(26)
L3	0%(0)	0%(0)	0%(0)	1%(1)	3%(2)	4%(3)	7(3)	7(3)	1%(1)	1%(1)
Hips										
L1	23%(9)	23%(9)	75%(45)	70%(42)	23%(9)	21%(8)	10%(2)	10%(2)	79%(54)	79%(53)
L2	78%(31)	78%(31)	25%(15)	30%(18)	74%(29)	74%(29)	76%(16)	76%(16)	19%(13)	21%(14)
L3	0%(0)	0%(0)	0%(0)	0%(0)	3%(1)	5%(2)	14%(3)	14%(3)	2%(1)	2%(1)
Knees										
L1	15%(5)	12%(4)	85%(46)	82%(44)	9%(3)	3%(1)	0%(0)	0%(0)	80%(41)	77%(39)
L2	85%(29)	88%(30)	15%(8)	17%(9)	88%(28)	94%(30)	100%(25)	100%(25)	20%(10)	24%(12)
L3	0%(0)	0%(0)	0%(0)	2%(1)	3%(1)	3%(1)	0%(0)	0%(0)	0%(0)	0%(0)
Responders										
All										
L1	0%(0)	100%(91)	0%(0)	96%(48)	0%(0)	90%(86)	0%(0)	76%(93)	0%(0)	92%(44)
L2	100%(91)	0%(0)	96%(48)	4%(2)	87%(83)	10%(100	54%(66)	24%(30)	88(42)	8%(4)
L3	0%(0)	0%(0)	4%(2)	0%(0)	14%(13)	0%(0)	46%(57)	0%(0)	13%(6)	0%(0)
Hips										
L1	0%(0)	100%(59)	0%(0)	95%(37)	0%(0)	89%(55)	0%(0)	85%(69)	0%(0)	91%(30)

L2	100%(59)	0%(0)	95%(37)	5%(2)	86%(53)	11%(7)	61%(49)	15%(12)	85%(28)	9%(3)
L3	0%(0)	0%(0)	5%(2)	0%(0)	15%(9)	0%(0)	40%(32)	0%(0)	15%(5)	0%(0)
Knees										
L1	0%(0)	100%(32)	0%(0)	100%(11)	0%(0)	91%(31)	0%(0)	57%(24)	0%(0)	93%(14)
L2	100%(32)	0%(0)	100%(11)	0%(0)	88%(30)	9%(3)	41%(17)	43%(18)	93%(14)	7%(1)
L3	0%(0)	0%(0)	0%(0)	0%(0)	12%(4)	0%(0)	60%(25)	0%(0)	7%(1)	0%(0)

3.2. EQ5D Non-Responders Exhibited Greater Pre-Operative Adiposity and Differential Concentrations of Peri-Operative Synovial Fluid Inflammatory Cytokines

We then compared baseline pre-operative characteristics including anthropometric data, disease severity and serum/synovial fluid cytokine between responder and non-responder patients. Compared to responder patients, non-responder patients had significantly greater BMI ($p<0.05$), waist circumference ($p<0.05$) and hip circumference ($p<0.05$). However, there was no difference in either KL grade or joint space between responder and non-responder patients suggesting that joint severity at the time of surgery was not a factor in post-operative outcomes (Table 3).

In total, we determined the concentration of 24 cytokines/adipokines in peri-operative synovial fluid and pre-operative serum, which have previously been reported to be associated with the inflammatory phenotype of OA [14-16]. There was no significant difference in the concentration of any of the 24 cytokines in the serum between responders and non-responder patients. However, comparison of peri-operative synovial fluid cytokine concentrations revealed that non-responders had significantly lower levels of Resistin (0.97 ng/ml vs 2.96 ng/ml, $p<0.05$) but significantly greater levels of leptin (54.5 ng/ml vs 16.3 ng/ml; $p<0.05$), compared to responders (Table 3).

Table 3. Baseline patient characteristics between EQ5Dindex responders and non-responders. All values are shown as median with IQR. ^=significantly different between responders and non-responders, $p<0.05$.

	All Patients		**Responders**		**Non-responders**	
Age (years)	70 (62,75)		70 (62, 75)		72.5 (63.5, 76.5)	
Height (cm)	166 (160, 175)		167 (160, 175)		163 (160, 173)	
Weight (kg)	75.7 (67.4, 91.7)		75.3 (66, 89.9)		81.4 (73, 93.7)	
BMI (kg/m^2)	27.3 (24.8, 30.7)		26.8 (24.6, 30,4)		*29.9 (27.5, 33.6)**	
WC (cm)	96.3 (85.3, 107)		96 (84, 105)		*108 (91, 112)**	
HC (cm)	107 (99, 113)		106 (99, 112)		*113 (104, 121)**	
WHR	0.91 (0.84, 0.96)		0.91 (0.84, 0.95)		0.92 (0.88, 0.98)	
Joint Space (mm)	0 (0,1)		0 (0, 1)		0 (0, 3.3)	
KL Grade	4 (3, 4)		4 (3, 4)		4 (2.8, 4)	
	Serum	*Synovial Fluid*	*Serum*	*Synovial Fluid*	*Serum*	*Synovial Fluid*
TNFα (pg/ml)	5.1 (4.2, 5.6)	5.1 (2.8, 9.5)	5.1 (4.2, 5.6)	5.1 (2.8, 9.5)	5.2 (4, 5.6)	5.1 (1.4, 14.0)

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Visfatin (ng/ml)	2.6 (1.8, 3.3)	0 (0, 32)	2.6 (1.8, 3.3)	0 (0, 36)	2.6 (1.8, 3.3)	0 (0, 0)
IL10 (pg/ml)	4.81 (4.46, 4.99)	17.3 (12.2, 22.6)	4.8 (4.46,4.99)	17.3 (11.5, 21.6)	4.7 (4.46, 5.05)	21.6 (16.1, 31.3)
IL1B (pg/ml)	15.9 (13.7, 17.8)	25.1 (9.6, 31.6)	15.9 (13.3, 17.8)	28.4 (9.0, 31.6)	16.1 (14.5, 18.2)	16.3 (7.2, 36.3)
DKK1 (ng/ml)	3.3 (2.3, 4.7)	0.50 (0.35, 0.71)	3.3 (2.3, 4.7)	0.48 (0.35, 0.72)	3.6 (2.2, 4.7)	0.53 (0.41, 0.84)
MIP1α (ng/ml)	0.42 (0.03, 0.54)	0.34 (0.24, 0.39)	0.42 (0.03, 0.53)	0.34 (0.24, 0.39)	0.46 (0.02, 0.60)	0.43 (0.16, 0.46)
gal1 (ng/ml)	43.9 (31.1, 58.0)	110 (87, 126)	40.7 (29.9, 57.7)	109 (86, 127)	53.4 (43.2, 67.8)	111 (90, 125)
Chemerin (ng/ml)	5.6 (3.4, 7.9)	3.0 (2.4, 3.6)	5.6 (3.5, 8.0)	3.0 (2.4, 3.6)	4.8 (2.7, 6.8)	3.3 (3.1, 4.0)
Eotaxin (pg/ml)	125 (61, 228)	28.9 (9.5, 42.6)	125 (62, 228)	28.9 (9.5, 42.6)	82 (61, 214)	28.9 (9.5, 42.6)
gp130 (ng/ml)	93 (69, 105)	71.4 (61.6 76.8)	93 (69, 104)	71.4 (61.5, 77.2)	102 (56, 108)	67.6 (58.9, 76.8)
ip10 (pg/ml)	23.4 (18.4, 32.4)	104.4 (70.7, 151.4	23.9 (18.4, 36.7)	106.3 (70.4, 152.9)	22.8 (17.4, 26.6)	98.9 (73.2, 265.1)
MCP1 (ng/ml)	0.35 (0.25, 0.46)	0.30 (0.15, 0.61)	0.35 (0.25, 0.47)	0.29 (0.15, 0.64)	0.35 (0.27, 0.41)	0.30 (0.18, 0.42)
IL7 (pg/ml)	2.7 (1.8, 3.9)	4.0 (2.8, 5.2)	2.7 (1.8, 3.4)	4.0 (2.8, 5.2)	2.9 (2.2, 4.4)	4.6 (4.0, 6.3)
MIP3α (pg/ml)	34.4 (8.8, 59.9)	22.7 (13.1, 42.6)	33.5 (8.8, 54.7)	22.9 (13.8, 43.7)	62.8 (7.1, 71.2)	9.4 (7.3, 42.1)
Amphiregulin (ng/ml)	0.59 (0.59, 0.59)	1.36 (0.55, 1.36)	0.59 (0.48, 0.59)	1.36 (0.55, 1.36)	0.59 (0.59, 0.59)	1.36 (0.78, 1.65)
IL15 (pg/ml)	4.0 (3.0, 4.7)	31.1 (20.9, 35.2)	4.0 (3.0, 4.9)	31.1 (18.8, 34.9)	3.4 (3.1, 3.8)	34.9 (27.0, 37.3)
Aggrecan (pg/ml)	163 (116, 217)	0 (0, 114)	163 (116, 217)	0 (0, 114)	116 (116, 204)	0 (0, 244)
Resistin (ng/ml)	14.5 (11.2, 18.3)	2.90 (1.72, 5.19)	14.5 (11.2, 19.0)	2.96 (1.99, 5.70)	14.9 (8.7, 16.2)	*0.97 (0.53, 2.65)**

Serpin E1 (ng/ml)	135 (100, 167)	15.6 (8.5, 42.7)	137 (105, 167)	16.2 (8.4, 49.4)	113 (19, 165)	11.0 (6.6, 18.3)
Adiponectin(ug/ml)	9.9 (6.34, 13.5)	2.7 (2.0, 4.6)	9.9 (6.8, 14.2)	2.8 (2.0, 5.3)	8.5 (1.4, 11.5)	2.5 (1.6, 3.2)
IL6 (pg/ml)	2.1 (0.0, 2.7)	132 (57, 453)	2.1 (0, 2.8)	175 (64, 577)	0 (0, 2.0)	69 (8, 222)
Leptin (ng/ml)	13.1 (6.7, 26.1)	17.3 (7.1, 54.6)	12.6 (6.3, 26.1)	16.3 (6.5, 48.1)	23.3 (12.7, 27.6)	*54.5 (40.3, 67.9)**
FABP4 (ng/ml)	18.0 (12.6, 28.2)	16.7 (8.2, 81.4)	17.9 (11.8, 27.2)	16.1 (7.7, 66.0)	18.8 (15.6, 41.0)	60.1 (11.7, 159.6)
MIP1β (ng/ml)	152 (105, 184)	55.9 (0, 105.3)	150 (101, 187)	55.9 (0, 105.3)	128 (85, 165)	0 (0, 80.6)

3.3. The Relationship between Pre-Operative Anthropometric Data and Post-Operative Change in EQ5D

Next, we investigated whether any of the baseline anthropometric and disease severity characteristics were predictive for post-operative outcome by performing linear regression analysis on their relationship to the change in EQ5Dindex (ΔEQ5D) between pre and 7 months post operation. Neither K/L grade nor joint space at the time of joint surgery was significantly related to ΔEQ5D. Furthermore, despite non-responders having on average a significantly higher BMI, waist circumference and hip circumference we did not observe a significant relationship between these variables and ΔEQ5D. However, baseline body weight showed a small but significant negative relationship to ΔEQ5D (β =-0.005, r=0.22, p=0.049), suggesting that high body weight at baseline explains a proportion of the poor post-operative response (Table 4).

Table 4. The relationship between anthropometric data and ΔEQ5D (pre and 7 month post-operatively)*.

	Linear regression coefficient (95% CI) †	**r**	**p-value**
AGE	-0.003 (-0.012, 0.006)	0.065	0.564
Height (cm)	-0.005 (-0.013, 0.003)	0.137	0.228
Weight (kg)	-0.005 (-0.009, -0.00002)	0.222	*0.049*
BMI	-0.012 (-0.026, 0.003)	0.181	0.108
Waist circumference (cm)	-0.003 (-0.007, 0.0007)	0.193	0.104
Hip circumference (cm)	-0.003 (-0.007, 0.002)	0.145	0.225
WHR	-0.456 (-1.35, 0.440)	0.121	0.313
Joint Space (mm)	-0.023 (-0.075, 0.029)	0.103	0.376

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K and L grade	0.015 (-0.077, 0.106)	0.037	0.751

*95% CI=95% confidence interval. † Change in EQ5D per unit increase in parameter.

3.4. The Relationship between Pre-Operative Serum and Synovial Fluid Cytokines and Post-Operative Change in EQ5D

We then examined whether the concentrations of pre-operative serum cytokines or peri-operative synovial fluid cytokines were predictive for the 7-month post-operative change in EQ5D (ΔEQ5D). Univariate linear regression was performed, followed by multivariate regression to account for potential confounders including joint severity (K/L grade, joint space), BMI, age, waist and hip circumference and body weight. Of the 24 cytokines quantified in the serum, we observed no significant relationship between cytokine concentration and ΔEQ5D, in either univariate or multivariate analysis (Table 5). Similarly, regression analysis of synovial fluid cytokines revealed no significant relationship between cytokine concentration and ΔEQ5D in either univariate or multivariate analysis with confounders (Table 6). However, there was a trend for a positive relationship between synovial fluid concentrations of IL-6 and ΔEQ5D (r=0.288, p=0.07) in univariate analysis, and a trend for a negative relationship between amphiregulin synovial fluid concentration and ΔEQ5D in both univariate (r=0.268, p=0.09) and multivariate analysis (r=0.619, p=0.07).

Table 5. Relationship between pre-operative serum cytokines and ΔEQ5D (pre and 7 month post-operatively)*.

	Linear regression coefficient (95% CI)†	**r**	**p-value**	**Multiple Linear regression coefficient (95% CI) ‡**	**r**	**p-value**
TNF-α	-0.006 (-0.04, 0.028)	0.046	0.715	-0.004 (-0.054, 0.046)	0.293	0.872
Visfatin	0.001 (-0.006, 0.07)	0.023	0.856	0.001 (-0.007, 0.008)	0.291	0.836
IL-10	-0.0003 (-0.005, 0.005)	0.013	0.92	-0.001 (-0.006, 0.005)	0.291	0.824
IL-1β	0.002 (-0.001, 0.006)	0.174	0.165	0.003 (-0.001, 0.007)	0.346	0.175
DKK1	-0.024 (-0.065, 0.017)	0.147	0.243	-0.031 (-0.082, 0.021)	0.344	0.239
MIP1α	0.042 (-0.223, 0.306)	0.04	0.754	0.014 (-0.307, 0.336)	0.292	0.928
Galectin1	-0.0008 (-0.003, 0.001)	0.097	0.442	-0.0001 (-0.003, 0.002)	0.292	0.932
Chemerin	-0.006 (-0.026, 0.013)	0.079	0.531	-0.007 (-0.032, 0.018)	0.301	0.586
Eotaxin	0.00014 (-0.00028, 0.00055)	0.082	0.519	0.00013 (-0.001, 0.001)	0.284	0.968
gp130	-0.0014 (-0.004, 0.001)	0.170	0.175	-0.002 (-0.004, 0.001)	0.336	0.230
IP10	0.00046 (-0.003, 0.004)	0.034	0.786	0.001 (-0.003, 0.005)	0.297	0.679
MCP1	0.004 (-0.059, 0.067)	0.017	0.895	0.028 (-0.049, 0.104)	0.308	0.472
IL-7	-0.018 (-0.076, 0.04)	0.079	0.531	-0.007 (-0.092, 0.078)	0.293	0.872
MIP3α	6.8×10^{-6} (-0.00013, 0.00014)	0.013	0.919	6.02×10^{-6} (-0.00016, 0.00014)	0.292	0.936
Amphiregulin	0.091 (-0.340, 0.522)	0.054	0.674	0.240 (-0.301, 0.780)	0.315	0.377

IL-15	0.002 (-0.012, 0.016)	0.034	0.788	0.001 (-0.016, 0.018)	0.290	0.920
Aggrecan	7.5x10^{-5} (-0.00014, 0.00029)	0.088	0.487	0.0004 (-0.0001, 0.001)	0.365	0.106
Resistin	0.007 (-0.001, 0.016)	0.219	0.085	0.006 (-0.005, 0.016)	0.329	0.288
Serpin E1	0.0005 (-0.0006, 0.0016)	0.110	0.383	0.001 (-0.001, 0.002)	0.318	0.361
Adiponectin	0.008 (-0.004, 0.020)	0.165	0.193	0.006 (-0.01, 0.022)	0.310	0.467
IL-6	1.9x10^{-5} (-0.001, 0.009)	0.005	0.970	-8.3x10^{-5} (-0.001, 0.001)	0.293	0.881
Leptin	0.00019 (-0.004, 0.004)	0.012	0.925	0.00014 (-0.007, 0.007)	0.279	0.967
FABP4	0.001 (-0.001, 0.004)	0.145	0.252	0.001 (-0.002, 0.004)	0.308	0.494
MIP1β	0.0006 (-0.0004, 0.0016)	0.209	0.102	0.001 (-0.001, 0.002)	0.312	0.430

*95% CI=95% confidence interval. † Change in EQ5D per unit increase in cytokine. ‡ Change in EQ5D per unit increase in cytokine including age, body mass index, Kellgren/Lawrence grade, joint space, waist and hip circumference, waist:hip ratio and body weight in the regression equation.

Table 6. Relationship between pre-operative synovial fluid cytokines and ΔEQ5D (pre and 7 month post-operatively)*.

	Linear regression coefficient (95% CI)†	**r**	**p-value**	**Multiple Linear regression coefficient (95% CI) ‡**	**r**	**p-value**
TNF-α	0.001 (-0.018, 0.020)	0.016	0.921	0.008 (-0.022, 0.037)	0.544	0.584
Visfatin	0.001 (-0.001, 0.003)	0.195	0.222	0.001 (-0.001, 0.004)	0.560	0.352
IL-10	-0.0004 (-0.010, 0.009)	0.012	0.940	-0.001 (-0.016, 0.013)	0.537	0.843
IL-1β	0.0005 (-0.006, 0.007)	0.024	0.881	0.002 (-0.006, 0.011)	0.544	0.589
DKK1	-0.073 (-0.225, 0.08)	0.153	0.339	-0.120 (-0.543, 0.302)	0.545	0.562
MIP1α	-0.284 (-0.882, 0.313)	0.152	0.342	-0.633 (-0.155, 0.283)	0.587	0.166
Galectin1	0.0018 (-0.0019, 0.0056)	0.156	0.329	0.0003 (-0.006, 0.005)	0.536	0.906
Chemerin	-0.023 (-0.099, 0.052)	0.100	0.534	0.004 (-0.117, 0.126)	0.536	0.940
Eotaxin	-0.0007 (-0.002, 0.001)	0.146	0.362	-0.001 (-0.002, 0.001)	0.545	0.559
gp130	-0.001 (-0.008, 0.006)	0.054	0.738	-0.003 (-0.013, 0.007)	0.545	0.565
IP10	0.00013 (-0.0005, 0.0007)	0.069	0.670	0.000019 (-0.001, 0.001)	0.535	0.960
MCP1	0.026 (-0.052, 0.105)	0.109	0.497	0.06 (-0.03, 0.155)	0.582	0.193
IL-7	-0.033 (-0.093, 0.028)	0.172	0.283	-0.031 (-0.112, 0.05)	0.533	0.438
MIP3α	0.002 (-0.002, 0.006)	0.164	0.307	0.001 (-0.005, 0.007)	0.541	0.654
Amphiregulin	-0.158 (-0.343, 0.026)	0.268	0.09	-0.24 (-0.5, 0.021)	0.619	0.07

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IL-15	-0.001 (-0.009, 0.008)	0.020	0.90	-0.003 (-0.016, 0.010)	0.543	0.615
Aggrecan	-0.0005 (-0.0015, 0.0005)	0.169	0.29	-0.0004 (-0.002, 0.001)	0.538	0.773
Resistin	0.0011 (-0.008, 0.010)	0.041	0.801	-0.002 (-0.016, 0.012)	0.538	0.777
Serpin E1	0.0018 (-0.0019, 0.0054)	0.154	0.338	0.001 (-0.006, 0.007)	0.537	0.808
Adiponectin	0.032 (-0.004, 0.069)	0.274	0.083	0.022 (-0.055, 0.098)	0.545	0.560
IL-6	0.0013 (-0.00001, 0.00028)	0.288	0.072	0.0001 (-0.00008, 0.0003)	0.569	0.223
Leptin	0.0002 (-0.0024, 0.0029)	0.028	0.868	0.0002 (-0.006, 0.006)	0.560	0.950
FABP4	0.00004 (-0.00009, 0.00018)	0.104	0.521	-0.001 (-0.003, 0.001)	0.552	0.348
MIP1β	0.00044 (-0.0012, 0.0021)	0.089	0.585	-0.001 (-0.003, 0.001)	0.566	0.239

*95% CI=95% confidence interval. † Change in EQ5D per unit increase in cytokine. ‡ Change in EQ5D per unit increase in cytokine including age, body mass index, Kellgren/Lawrence grade, joint space, waist and hip circumference, waist:hip ratio and body weight in the regression equation.

Exploring this further, we examined using multiple regression whether the combination of body weight with amphiregulin and/or IL-6 peri-operative synovial fluid concentration would provide a better predictor of ΔEQ5D (Table 7). Amphiregulin synovial fluid concentration with body weight was a significant predictor for the post-operative change in EQ5D (r=0.434, p=0.023), with the equation ΔEQ5D = -0.108[Amphiregulin ng/ml] -0.0006[Body weight kg] + 1.052. Similarly, IL-6 synovial fluid concentration with body weight was also a significant predictor for the post-operative change in EQ5D (r=0.418, p=0.035), with the equation Δ EQ5D = 7.4×10^{-5}[IL-6 pg/ml] -0.006[Body weight kg] + 0.871. Finally, the combination of both amphiregulin and IL-6 synovial fluid concentrations with body weight provided a marginal improvement in the relationship (r=0.470, p=0.035) with the equation Δ EQ5D = 8.3×10^{-5}[IL-6 pg/ml] -0.142[Amphiregulin ng/ml] -0.005[Body weight kg] + 0.919.

Table 7. Multiple regression models.

	r	**p-value**	**Unstandardized Coefficients (B)**			**Equation**
			Cytokine	**BW**	**Constant**	
Amph, BW	0.434	*0.023*	-0.108	-0.0006	1.052	ΔEQ5D = -0.108 [Amph ng/ml] - 0.0006 [BW kg] + 1.053

IL-6, BW	0.418	*0.035*	$7.4x10^{-5}$	-0.006	0.871	ΔEQ5D = $7.4x10^{-5}$ [IL-6 ng/ml] - 0.006 [BW kg] + 0.871
Amph, IL-6, BW	0.470	*0.035*	-0.142 (Amph) 8.3x10-5 (IL-6)	-0.005	0.919	ΔEQ5D = -0.142 [Amph ng/ml] +$8.3x10^{-5}$ [IL-6 ng/ml] -0.005 [BW kg] + 0.919

Amph= Amphiregulin, BW=Body weight.

4. Discussion

This is the first study to profile pre-operative and peri-operatively 24 serum and synovial fluid cytokines and to analyse their associations with post-operative outcomes in knee and hip OA patients following joint replacement surgery. Based on change in EQ5D index we identified a population of knee and hip OA patients as non-responders with little to no improvement in any of the 5 individual EQ5D components including mobility, self-care, usual activities, pain/discomfort and anxiety/depression.

The comparison between the responder and non-responder groups revealed that on average the non-responders were of greater adiposity, with higher BMI and waist and hip circumferences. In line with this observation, non-responders also had significantly higher levels of the obesity-associated adipokine leptin in their joint synovial fluid at the time of joint replacement surgery. Interestingly we observed no difference in the concentration of leptin in the pre-operative serum between responders and non-responders, suggesting that it is the local effect of adiposity on the joint that is more relevant to post-operative outcomes. Taken together these observations infer that the obesity phenotype is associated with poor post-operative outcomes. Notably, obesity is a known risk factor for OA [17, 18], and recently it was determined via molecular endotyping that obesity impacts the inflammatory synovial fibroblast phenotype of not only load-bearing (e.g. knees and hips) [14, 15] but also non-load bearing joints such as the hands [19]. Furthermore, we and others have previously reported that obesity impacts the phenotype of multiple tissues within the synovial joint, including cartilage [20], subchondral bone [21] and the skeletal muscle [22, 23]. This is in line with recently published meta-analysis which demonstrated that pre-surgical obesity was associated with worse clinical outcomes of joint replacement procedure [24]. Furthermore, peri-operative levels of synovial fluid leptin were previously reported to significantly correlate with pre-operative level of pain reported in patients with hip OA [5].

In attempting to establish a predictive model for post-operative outcomes, we performed linear regression analysis of baseline anthropometric and cytokine profiles with change in EQ5Dindex from pre to post-operation. Firstly, our analysis found no relationship between pre-operative K/L grade or joint space with ΔEQ5D, suggesting that disease severity at the time of surgery does not impact on likely outcome. Our finding that body weight was significantly but negatively related to ΔEQ5D provides further support to the notion that increased adiposity is a likely predictor of poor post-operative outcomes. Despite not finding a significant relationship between ΔEQ5D and either the serum or synovial fluid concentration of any one individual cytokine, the synovial fluid concentrations of amphiregulin and/or IL-6 in combination with body weight provided a model that could explain a proportion of the ΔEQ5D response. The lack of a strong relationship between any one individual cytokine and ΔEQ5D suggests that a highly accurate and predictive model is most likely to be developed through the combination of multiple biomarkers, including anthropometric data.

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Furthermore, our data would suggest that the pre-operative concentration of cytokines in the joint synovial fluid will provide better predictive biomarkers than serum cytokine concentrations.

This study has some limitations. Firstly, expansion of the panel of 24 cytokines/adipokines included in our study would provide a more comprehensive cytokine profile related to mediating pain, which could be informative. Secondly, this study represents a relatively small cohort of 160 number of hip and knee OA patients and therefore requires validation in a larger cohort. However, the observed difference we report in the proportion of positive outcomes between knee and hip joint replacements has been reported previously [25-27] and thus suggests that the dataset is representative of larger dataset studies. Finally, the absence of post-operative levels of cytokine and adipokines means that we cannot determine the impact of post-operative cytokines on outcome.

5. Conclusions

In conclusion, a predictive model combining the synovia fluid concentrations of the cytokines amphiregulin and IL-6 with body weight pre-operatively could explain a proportion of the post-operative change in EQ5Dindex health status. Further analysis of pre-operative markers and validation across a larger patient cohort is required in order to develop a highly accurate and predictive model that could have utility for both clinicians and their patients.

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