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Lymphatic vessels in osteoarthritic human knees

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SUMMARY

Objectives: The distribution and function of lymphatic vessels in normal and diseased human knees are understood incompletely. This study aimed to investigate whether lymphatic density is associated with clinical, histological or radiographic parameters in osteoarthritis (OA).

Methods: Sections of synovium from 60 knees from patients with OA were compared with 60 post mortem control knees (from 37 individuals). Lymphatic vessels were identified using immunohistochemistry for podoplanin, and quantified as lymphatic vessel density (LVD) and lymphatic endothelial cell (LEC) fractional area. Effusion status was determined by clinical examination, radiographs were scored for OA changes, and inflammation grading used haematoxylin and eosin stained sections of synovium.

Results: Lymphatic vessels were present in synovia from both disease groups, but were not identified in subchondral bone. Synovial lymphatic densities were independent of radiological severity and age. Synovia from patients with OA displayed lower LVD ($z = -3.4$, $P = 0.001$) and lower LEC fractional areas ($z = -4.5$, $P < 0.0005$) than non-arthritic controls. In patients with OA, low LVD was associated with clinically detectable effusion ($z = -2.2$, $P = 0.027$), but not with histological evidence of synovitis. The negative associations between lymphatics and OA/effusion appeared to be independent of other measured confounders.

Conclusion: Lymphatic vessels are present in lower densities in OA synovia. Abnormalities of synovial fluid drainage may confound the value of effusion as a clinical sign of synovitis in OA.

Introduction

Synovial pathology is increasingly recognised as a potential target for treatment in osteoarthritis (OA). Synovial inflammation and effusion may contribute to symptoms and progressive joint damage in OA. Lymphatic vessel abnormalities may contribute to inflammation and tissue fluid accumulation, but have previously attracted little attention in the osteoarthritic synovium.

Lymphatics are responsible for tissue fluid clearance and the transit of macromolecules to lymph nodes, where they may stimulate specific immune responses. Lymphatics are present in human[1–5] and rodent[6–8] synovium, accompanying arterioles and venules. Functionality of the articular lymphatic system has been demonstrated as clearance of macromolecules from synovial cavity into draining lymphatics[9,10]. However, changes that may occur in lymphatic vessels during arthritis remain incompletely understood.

Lymphangiogenesis, the growth of new lymphatic vessels, is regulated by factors such as vascular endothelial growth factors (VEGF)-C and D through the receptor VEGFR-3[11]. Lymphangiogenesis is increased during wound healing, inflammation and neoplasia. Inflammation can stimulate lymphangiogenesis through the upregulation of VEGF-C, for example in synovitis[7,12], and increased abundance of Lymphatic Vessel Endothelium receptor-1 (LYVE-1) immunoreactive vessels have been reported in the superficial regions of inflamed synovia[2].

In contrast to the expected increase in lymphangiogenesis in inflamed synovium, OA has also been associated with deficient fluid clearance. Presence or absence of synovial effusion, although often used clinically as a sign of inflammation, is a poor predictor of response to anti-inflammatory treatments such as intra-articular corticosteroids in OA[13]. Normally, trans-synovial flow is well maintained to preserve fluid homeostasis and prevent joint swelling. In joints with chronic effusions, this flow may exceed capacity for lymphatic drainage[10,14]. Furthermore, clearance of macromolecules is low from OA synovial cavities suggesting dysregulated lymphatic function[10].

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Lymphatic distribution in joint tissues other than the synovium has not been explored in detail. Normal bone marrow is devoid of lymphatics, with fluid clearance being through venous sinusoids within the marrow cavities. However, subchondral bone marrow may be replaced by fibrovascular tissue in OA[15] and, with the loss of integrity of the osteochondral junction, subchondral bone may also provide a route of fluid clearance from the synovial cavity[16].

Interpretation of previous studies of lymphatic vessels in joint tissues is hampered by small case numbers, control tissues with limited clinical characterisation and qualitative methodologies. Antigenic markers that are normally specific for lymphatic endothelium, may cross react with blood vessel endothelium in inflamed tissues[12,17] and loss of specificity of markers may have contributed to the apparent absence of lymphatics in some studies of joint tissues[1]. Lymphatics comprise <5% of total (lymphatic and blood) vessels in human synovium, and, given the established increase in synovial angiogenesis during inflammation[18,19], any cross reactivity of reagents with blood vessels may artificially create an impression of increased lymphatic density.

Podoplanin is a mucin-type transmembrane glycoprotein that is a commonly used lymphatic marker as it is expressed by lymphatic endothelial cells (LECs), and possibly some macrophages[20], but not by blood vascular endothelium[21–23]. It co-localises with VEGFR-3, and is expressed on Pathologische Anatomie Leiden-Endothelium (PAL-E) negative vessels, reinforcing its specificity for lymphatic vessels[24].

A fuller characterisation of lymphatic distribution in OA is important in establishing any potential roles for lymphatics in regulating inflammation and clearing tissue fluid. This study aimed to investigate the distribution of lymphatics in normal and arthritic human knees, to quantify any differences in lymphatic densities between disease groups, and to explore any possible relationships between variations in lymphatic density, synovitis and joint effusion status. We hypothesise that a decrease in the number of lymphatic vessels in the osteoarthritic synovium leads to decreased drainage of synovial fluid from the joint, thereby contributing to joint effusion.

Materials and methods

Sample collection and processing

The study was approved by the National Research Ethics Service, reference numbers 05/Q2403/24, 05/Q2403/61, NNHA/420, NNHA/405, NNHA/473 and NN/544. All those who had donated tissue had provided informed consent as per the Declaration of Helsinki.

Archival material in the Division of Academic Rheumatology was collected at Sherwood Forest Hospitals NHS Foundation Trust, Sutton in Ashfield, UK. Each OA sample was taken during a total knee replacement arthroplasty from patients who satisfied American College of Rheumatology criteria for the diagnosis of knee OA[25]. Post mortem tissues were processed identically to surgical samples after harvesting by an experienced technician. Synovium from adjacent to the medial tibial plateau, and mid-coronal slices of medial tibial plateaux were fixed in formalin. These sites were selected based on the higher prevalence and severity of OA in the medial tibial plateau, and the a priori hypothesis that synovium is more likely to influence and be influenced by adjacent bone and cartilage than is that at more distant locations. Medial tibial plateaux were immersed in ethylenediaminetetraacetic acid (EDTA) at room temperature until decalcification was confirmed by radiography. Samples were wax-embedded and 5 μm sections used for histological analysis.

Sample selection

An initial pilot study of OA ($n = 15$), rheumatoid arthritis (RA) ($n = 16$) and post-mortem ($n = 15$) tissues suggested that lymphatic densities might be lower than normal in OA synovia. A power calculation[26] based on these data indicated a required sample size of 52 synovia per group to give 80% power of detecting the observed difference between OA and PM as significant at the 5% level.

Samples were purposively selected for this study in order to minimise the likelihood of joint disease in post mortem controls, and to permit attribution of findings to OA rather than demographic status, given the strong association of OA prevalence with age and gender. Sixty post mortem knees were selected from 37 cases. Post mortem knees were excluded from patients who were known to have OA or other arthropathy, if Heberden's nodes were identified by clinical examination, or if either osteophytes (OST) or grade III or IV chondropathy was apparent on macroscopic examination of tibial plateaux and femoral condyles[27]. Sixty OA cases were selected, one OA case age matched within 6 years to each post mortem knee. Where more than one age-matched sample was available in the archive, OA cases were selected to be matched by gender, and by age closest to the PM case.

Synovia from all 120 knees (60 OA and 60 post mortem knees) were used for analysis of podoplanin immunoreactivity, as per our original protocol. Analyses were repeated using only one knee, randomly selected from each of the 37 post mortem cases in order to ensure that unknown intra-person factors did not invalidate statistical presumptions about independence of observations. A subgroup of 20 cases, comprising 10 OA and 10 post mortem cases, was selected for further evaluation of podoplanin immunoreactivity in subchondral bone. A subgroup of nine cases, comprising six OA and three post mortem cases, was selected for evaluation of LYVE-1 and CD34 immunoreactivities, and possible associations with podoplanin-immunoreactivity determined by staining of consecutive tissue sections.

Histochemistry

Haematoxylin and eosin-stained sections were graded for the presence of inflammatory cells, cellularity and synovial thickness, as previously described[18]. Possible grades were 0; normal (synovial lining <4 cells thick, sparse cellular distribution, with few or no inflammatory cells), 1; mild inflammation (synovial lining four or five cells thick, increased cellularity with some inflammatory cells), 2; moderate inflammation (synovial lining six or seven cells thick, dense cellularity with inflammatory cells but not lymphoid aggregates), 3; severe inflammation (synovial lining >7 cells thick, dense cellularity and inflammatory cell infiltration, may contain perivascular lymphoid aggregates).

Immunohistochemistry used monoclonal antibodies to podoplanin at a dilution of 1:100, or LYVE-1 at a dilution of 1:500, or CD34 at a dilution of 1:100, each for 60 min, then biotinylated horse anti-mouse antibodies for 30 min, avidin–biotin–alkaline phosphatase complex for a further 30 min and developed using *FastRed*™. All incubations were at room temperature. All sections from both patient groups were stained within a single staining run in order to minimise technical variance.

Image analysis

Synovial sections stained for podoplanin were viewed through a ×20 objective lens and the four fields of view with the highest lymphatic vascular densities in each section were quantified using a KS300 computer-assisted image analysis system (Imaging Associates, Bicester, UK). Synovium was delineated interactively, and podoplanin-positive vessels were defined by interactive thresholding according to hue. Lymphatic vessel profiles were identified as round or linear podoplanin-positive structures surrounded by podoplanin-negative tissue. This method is comparable to previous

methodology for quantifying vascular endothelium by us[18,28] and synovial lymphatics by others[5]. All histomorphometry was undertaken by an observer blinded to diagnostic, clinical and radiographic status.

Effusion status

Effusion status in the operated knee for OA cases was determined as present or absent by one observer (D. Wilson) during a pre-operative clinical examination, using the balloon sign[29].

Radiograph scoring

Pre-operative postero-anterior radiographs for OA cases were graded by an observer blinded to histological findings, as an index of structural disease severity. Joint space narrowing (JSN) and presence of OST were graded by comparison with a line drawing atlas, with possible ranges from 0 to 6 and 0 to 12 respectively and higher scores indicating more severe structural change[30]. These individual scores were summed to give a total severity score out of a possible 18. No radiographs were taken from post mortem patients.

Data analysis

Lymphatic Vascular Density (LVD) was defined as the number of podoplanin-positive lymphatic vessel profiles per mm^2 of synovium in the four fields of view. LEC fractional area was measured as the thresholded podoplanin-positive vessel area per mm^2 of synovium in the four fields of view. Podoplanin-immunoreactive structures that did not display vascular morphology, when present, were interactively excluded. Spaces not containing tissue matrix or cells were excluded from synovium area measurements to avoid potential confounding by *ex vivo* and tissue processing artifacts, in particular the extent of lymphatic dilation.

Data were analysed with Statistical Package for Social Sciences, version 14 (SPSS Inc, Chicago, IL, USA), using Mann–Whitney *U*-tests, Spearman's rank correlation coefficients or χ^2 statistic. Further statistical analysis was performed using logistic regression to attempt to control for possible confounders, and for this analysis continuous data were divided into quartiles. Age, gender and histological inflammation score, plus any factors found in univariate analyses to be significantly associated with the dependent variable, were defined as potential confounders prior to the study. $P < 0.05$ indicated statistical significance. Data are presented as median and interquartile range (IQR).

Materials

Monoclonal antibody anti-podoplanin (cat# 11-003)[31] came from AngioBio, Del Mar, CA, USA. Monoclonal antibodies against LYVE-1 (clone 537028; cat# MAB20892) or CD34[15] (clone QBEnD10; cat# M7165) came from R&D Systems and Dako respectively. Biotinylated horse anti-mouse immunoglobulin gamma (IgG) antibody and avidin–biotin complex (ABC) alkaline phosphatase came from Vector Laboratories Ltd., Peterborough, UK. Except as stated, all other reagents were from Sigma–Aldrich, Poole, UK.

Results

Patient details

Demographics, clinical and radiographic characteristics are summarised in Table I. The age and gender of both sample groups were similar due to matching. Radiographic OA severity scores indicated severe structural change in OA patients, whereas post mortem cases had been selected to exclude possible subclinical OA.

Table I
Characteristics of the study population

	OA	PM
Patient numbers	60	37
Female	43%	46%
Median (IQR) age	63 (50–69)	63 (50–69)
Finger nodes (≥3)	10%	0%
JSN (range 0–6)	5 (IQR 4–5)	Not available
OST score (range 0–12)	8 (IQR 8–10)	Not available
Total radiographic score (range 0–18)	13 (IQR 12–15)	Not available
On medications for diabetes mellitus	2%	3%

The characteristics of each group are shown.

Lymphatic vessel localisation

Podoplanin-positive vessels were found in the deep synovium in OA and post mortem cases, usually clustered together near blood vessels [Fig. 1(A)]. Vessels containing erythrocytes were never positively stained for podoplanin, supporting the specificity of podoplanin for lymphatic endothelium. Consecutive tissue sections demonstrated that podoplanin-positive vessels were CD34-negative, and CD34-positive blood vessels were podoplanin-negative [Fig. 1(A, B)]. By contrast, LYVE-1 positive vessels were often associated with CD34 immunoreactivity, and sometimes displayed thick walls consistent with staining of blood vessels [Fig. 1(C, D)]. CD34-positive blood vessels were localised to both superficial and deep regions of the synovium, and appeared more abundant than were podoplanin-positive vessels. Podoplanin-positive vessels were not identified in subchondral bone, despite the presence of podoplanin-positive vessels in synovium attached to osteochondral tissues and despite the replacement of subchondral bone marrow by fibrovascular tissues (data not shown).

Lymphatic Vessel Density

Synovia were assessed from 60 knees per group, taken from 60 patients with OA and 37 post mortem cases. In order to reduce heterogeneity due to factors other than disease group, OA and post-mortem cases were age-matched, and inclusion of OA cases in the PM group was minimised by application of rigorous exclusion criteria.

Synovia from patients with OA displayed lower LVD (median 12 (IQR 0–20) mm^{-2}) and lower LEC fractional areas (median 0.6 (IQR 0–1.7)%) than PM samples (median lymphatic vessel density (LVD) 19 (IQR 13–26) mm^{-2}, $z = -3.4$, $P = 0.001$, median LEC fractional area 2.4 (IQR 1.3–3.8)%, $z = -4.5$, $P < 0.0005$, Fig. 2(A, B)).

41% (24/58) OA cases had clinically detectable knee joint effusion (data were missing for two cases). OA synovia from patients with effusion had lower LVD (median 0 (IQR 0–14) mm^{-2}) than OA cases without (median 15 (IQR 2–22) mm^{-2}, $z = 2.2$, $P = 0.027$, Fig. 2(C)). Difference in LEC fractional area between cases with and without effusion did not reach statistical significance (median 0.0 (IQR 0.0–1.7)% and 1.0 (IQR 0.1–1.7)% respectively, $z = 1.7$, $P = 0.081$, Fig. 2(D)). No significant associations were found between radiographic severity scores and LVD or LEC fractional area (see Supplemental Data, Table SI).

Synovial inflammation grade was higher in OA (median = 3, IQR: 0–3) compared to PM (median 1 (IQR 0–2) ($z = -2.6$, $P = 0.009$) (Fig. 3)). Synovial inflammation grades were similar in cases with or without effusions (median 2 (IQR 0–3) and 3 (IQR 0–3) respectively, $z = 1.0$, $P = 0.308$). Inflammation grades were

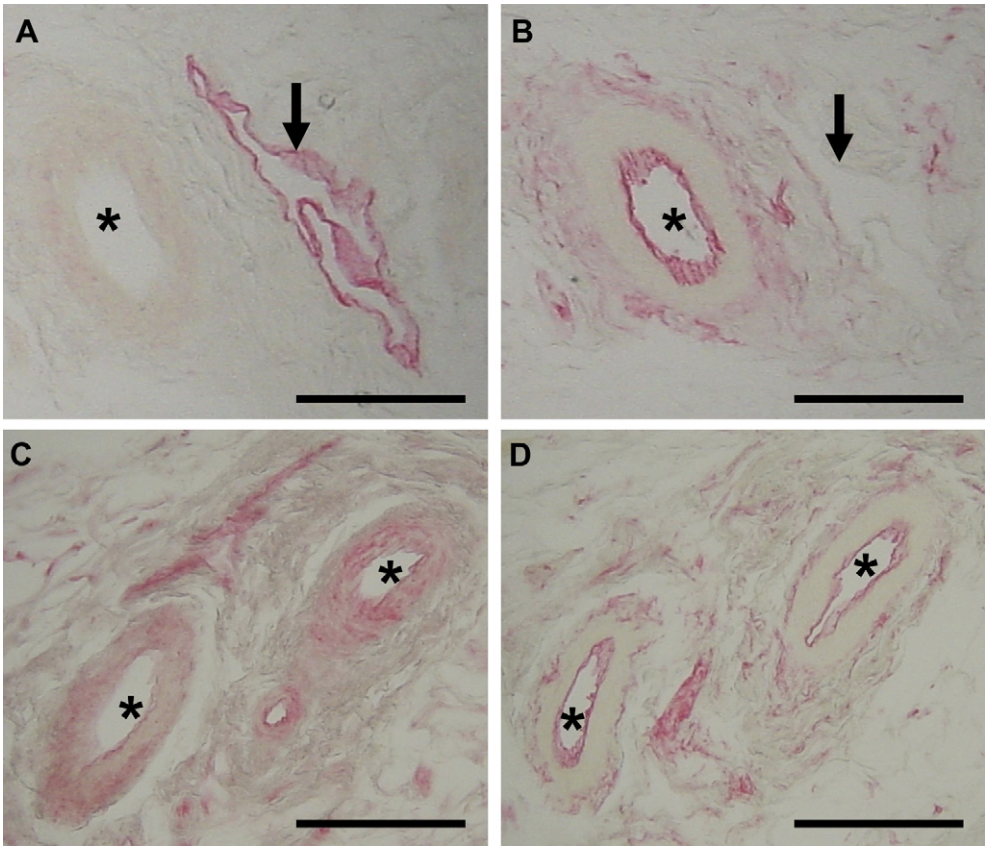

Fig. 1. Spatial relationships between lymphatic markers and CD34-positive blood vessels in human synovium. **A**: Podoplanin immunoreactivity (A) is present on a lymphatic vessel (arrow), but not on an adjacent blood vessel (asterisk). **B**: CD34 immunoreactivity in a tissue section consecutive to that shown in (A) is demonstrated on blood vessel endothelium, but not on the adjacent lymphatic vessel. CD34 positive blood vessels were more abundant than podoplanin-positive lymphatic vessel. **C**: Immunoreactivity for LYVE-1 is demonstrated on vascular structures (asterisks) that also display CD34-immunoreactivity in a consecutive section (**D**). Bar = 100 μm.

not significantly associated with LVD ($r = -0.09$, $P = 0.327$), nor with LEC fractional area ($r = -0.12$, $P = 0.178$).

Findings from univariate analyses were supported by logistic regression analysis. In comparisons between disease groups, LVD and LEC fractional areas were negatively associated with OA after adjusting for age, gender and inflammation (Table II). Random selection of one knee for each post-mortem case (giving $n = 37$) did not alter conclusions from either univariate or logistic regression analyses (Supplementary data tables SII and SIII respectively). Also, LVD was negatively associated with effusion in the patients with OA after adjusting for age, gender, inflammation and radiographic score (Table III). Synovial inflammation was associated with OA, but not associated significantly with effusion in these analyses.

Discussion

We found that synovial lymphatic density was reduced in patients with OA undergoing total knee replacement surgery, compared to non-arthritic controls, and this was associated with the presence of a clinically detectable effusion, but not with histological evidence of synovitis.

Lymphatics play key roles in the clearance of tissue fluid and the trafficking of immune cells in soft tissues. Despite this, study of lymphatics in human joint tissues has been limited by a paucity of specific reagents for their localisation, and limited availability of joint tissues. In contrast to the current consensus on increased blood vessel angiogenesis in OA, synovial lymphatics have been reported to be either absent[1] or increased[2] in arthritic human synovia. Our data indicate that podoplanin-positive lymphatic vessels are present in both normal synovium, and in OA synovia. By studying a large number of non-arthritic knees we have been able to demonstrate that lymphatic vascular densities are reduced in OA compared with non-arthritic synovium.

Xu *et al* have reported numerous lymphatic vessels close to the intimal lining in inflamed human synovia[2]. We found that podoplanin-positive lymphatic vessels were localised to deep regions of both normal and OA synovium, irrespective of the extent of synovitis, although this does not preclude the possibility that a subgroup of lymphatic vessels were not detected by our methods. Another study of normal synovial lymphatics also reported deep localisation, with vessels close to the synovial lining being exclusively positive for the blood vessel marker PAL-E[1]. Blood vessels are much more abundant in human synovium than are lymphatic vessels, and even a small cross-reaction of reagents between lymphatic and blood vessels might create a false impression of increased lymphatic density. Markers such as LYVE-1 and VEGFR3, although specific for lymphatics in normal tissues, may be upregulated by blood vessel endothelium during inflammation[12,17]. LYVE-1 was found in some samples in the current study to label CD34 positive blood vessels in addition to lymphatics, whereas podoplanin-positive lymphatics were CD34 negative. These findings, together with the thin-walled morphology of podoplanin-positive vessels, are consistent with podoplanin being a specific marker of synovial lymphatic vessels, even in inflamed osteoarthritic synovium.

Other studies have indicated that podoplanin may also be expressed by macrophages in some tissues[32,33]. Podoplanin-immunoreactive macrophages were not identified even in

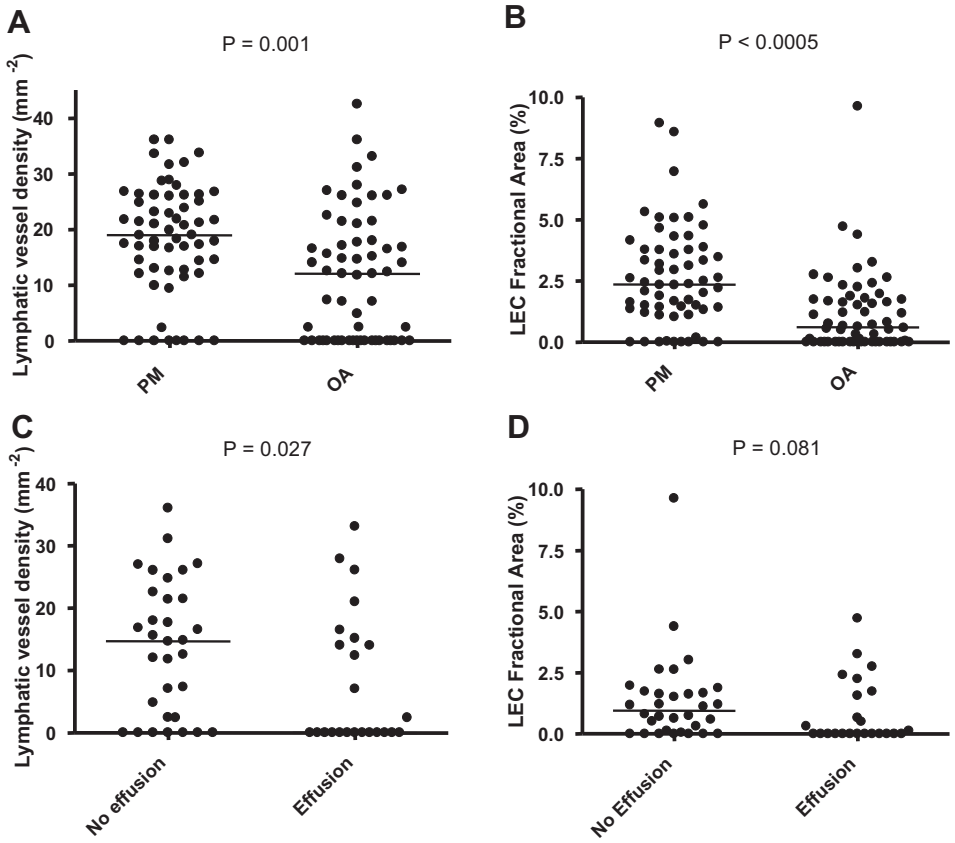

Fig. 2. Lymphatic densities in knee synovia. Data represent individual synovia and median values for each disease group. Lymphatic densities were lower in OA than in PM controls, both measured as lymphatic vascular density (**A**, $z = -3.4$, $P = 0.001$) and as lymphatic endothelial cell (LEC) fractional area (**B**, $z = -4.5$, $P < 0.0005$). Synovia from patients with OA displayed lower lymphatic vascular densities in cases in which a joint effusion was present than when absent (**C**, $z = -2.2$, $P = 0.027$), although difference in LEC fractional area between cases with and without effusion did not reach statistical significance (**D**, $z = -1.7$, $P = 0.081$). PM; post mortem controls, OA; osteoarthritis. Graphs are drawn to different scales.

inflamed OA synovia in the current study, although we could not rule out by light microscopy their possible presence closely adjacent to lymphatic vessels[34].

Our current data support previous findings by ourselves and others that synovitis is a feature of OA synovium[27,35]. Synovitis, as scored using histology in the current study, is indicative of increased cellularity. High scores in our OA cases indicate that low lymphatic densities are not due to replacement of synovium by inactive, fibrotic tissue in late stage disease. Increases in blood vessel density in synovia are associated with histological evidence of synovitis, an association attributed to the production of angiogenic factors such as VEGF[28,36]. Inflammation, for example during wound healing, can also stimulate lymphangiogenesis, through upregulation of VEGF-C[37,38] and previous studies have indicated

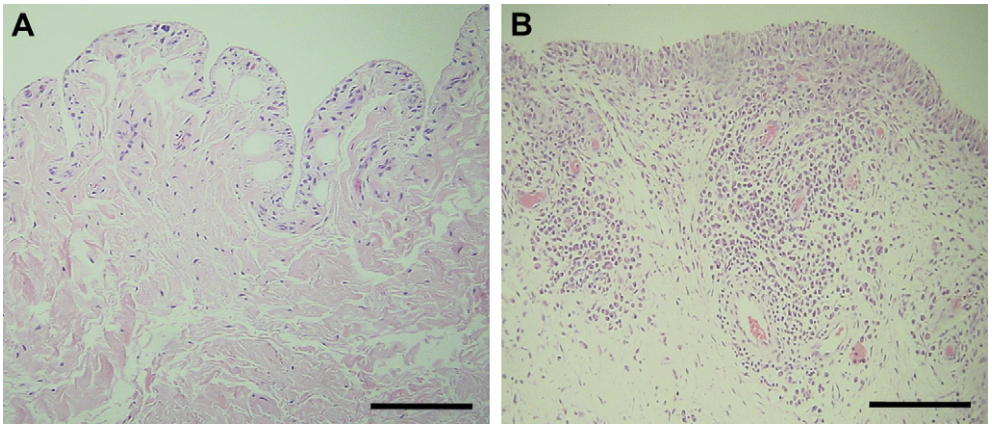

Fig. 3. Histological appearances of human knee synovium. Photomicrographs showing representative samples of OA and non-arthritic synovium displaying synovitis scores that are median for their respective disease group. **A**: Non-arthritic post mortem control displaying grade 1 inflammation score. **B**: OA synovium displaying grade 3 inflammation score, characterised by increased cellularity, infiltration by inflammatory cells, and increased depth of lining cell layer. Haematoxylin and eosin stains. Bar = 100 μm.

Table II
Logistic regression models of the association between OA and lymphatics

OA vs post mortem		LEC fractional area model		LVD model	
		aOR (95% CI)	*P*	aOR (95% CI)	*P*
Female	Y/N	1.01 (0.43–2.39)	0.983	0.90 (0.39–2.07)	0.795
Age	Quartile	0.96 (0.66–1.40)	0.837	1.05 (0.73–1.53)	0.787
Inflammation	0–3	1.63 (1.15–2.30)	0.006	1.60 (1.14–2.25)	0.006
Lymphatic endothelial area	Quartile	0.51 (0.35–0.75)	0.001	Not included	
LVD	Quartile	Not included		0.59 (0.41–0.86)	0.005

Independent associations with OA (adjusted for other factors in the model) were investigated using two logistic regression analyses; one for each measure of lymphatics in synovia. In both models, lymphatics showed a negative association with OA as the adjusted odds ratios (aOR) and 95% confidence intervals (CI) were significantly less than unity. Inflammation grade was positively associated with OA, and the other measures showed no significant relationship in this study.

that lymphatic vessel densities may be increased in inflamed synovium[2]. Despite this, lymphatic vascular densities were not significantly associated with synovitis in our cases, and were decreased rather than increased in OA synovia. This may suggest that synovial blood and lymphatic vessel densities are differentially regulated in OA. Low lymphatic densities may indicate that lymphangiogenesis is unable to keep pace with synovial hyperplasia or oedema, or may be due to damage to the pre-existing lymphatics during disease. VEGF-C and its receptor VEGFR-3 are expressed in human synovium[12], but do not appear to be sufficient to maintain lymphatic densities in OA. Although reduced lymphatic vessel densities in OA synovium were associated with clinical effusion status, we are unable to determine possible moderating effects of changes in total synovial lymphatic mass, which depend also on synovial tissue oedema and hyperplasia.

Joint effusion has been associated with OA radiographic severity[39] and pain[40], and has been used as a sign of inflammation. Although synovitis may lead to increased synovial fluid production[41], the presence of a clinically detectable synovial effusion in our study was not associated with histological evidence of synovitis, but rather with a reduced density of synovial lymphatics. The presence of synovial effusion represents an imbalance between the production and clearance of synovial fluid from the joint cavity. This leads us to suggest that effusion may be more indicative of lymphatic dysregulation in patients with OA, rather than of inflammation. This may partly explain the poor predictive value of clinical effusion for response in OA to intra-articular corticosteroid injection[13].

Our data support the findings of others[42] that subchondral bone spaces remain devoid of lymphatics even in advanced OA at the time of joint replacement surgery, and when fibrovascular tissue has replaced the subchondral bone marrow. Subchondral and synovial inflammation may each contribute to symptoms in OA[15,43], Differences between these tissue compartments in tissue fluid drainage and lymphocyte trafficking pathways may point to differences in the generation and resolution of oedema and immune responses between these tissue compartments.

Attribution of findings to the presence of OA can be compromised through confounding by other factors known to be strongly associated with OA prevalence and severity. In particular, increasing age and female gender have been associated with knee OA. We avoided potential confounding first by matching between OA and non-arthritic PM groups, and second by demonstrating that our findings were independent of age and gender. This approach requires selection of cases from a much larger database[44]. The Academic Rheumatology tissue repository currently contains knee samples from >2,000 cases collected at total joint replacement surgery, and >250 cases collected post mortem. Matching OA cases against non-arthritic PM cases produced a disease group that was slightly younger than other local studies that recruited from joint replacement lists[45], and a higher proportion of OA cases were males than would be representative of the local community[46]. Our method of case selection may have reduced our ability to find significant associations between LVD and age or gender. Further studies with access to a larger group of elderly non-arthritic patients would be required to confirm whether differences in lymphatic vessel densities persist with increasing age.

Single time point studies such as ours are subject to several limitations because we were studying late-stage tissue, and cannot show temporal associations. Interventional and longitudinal studies would be required to confirm whether the observed reduction in lymphatic densities in OA is a consequence of OA, and whether it contributes to the disease progression. As in previous studies[5], we used a method of 'hot spot' quantification, which reduces measurement error attributable to low lymphatic vessel densities. All histological studies require sampling from the total synovium and consistent reporting of sampling procedure is important in interpreting results. We have previously demonstrated associations between histological synovitis in synovium from adjacent to the medial tibial plateau and increased synovial blood vessel growth[34] and OA structural pathology[27]. These associations, as well as that with clinical effusion reported here, may indicate a pathological importance for this synovial region in OA. Further research would be required to determine whether

Table III
Logistic regression models of the association between effusion and lymphatics in OA

Effusion vs no effusion		LEC fractional area model		LVD model	
		aOR (95% CI)	*P*	aOR (95% CI)	*P*
Female	Y/N	1.10 (0.32–3.81)	0.886	1.05 (0.29–3.78)	0.945
Age	Quartile	0.66 (0.36–1.23)	0.189	0.66 (0.35–1.25)	0.199
Radiographic score	0–18	0.96 (0.81–1.12)	0.579	0.97 (0.83–1.15)	0.750
Inflammation	0–3	0.77 (0.48–1.25)	0.291	0.75 (0.45–1.23)	0.249
Lymphatic endothelial area	Quartile	0.56 (0.30–1.06)	0.077	Not included	
LVD	Quartile	Not included		0.49 (0.26–0.92)	0.025

Independent associations with effusion in patients with OA (adjusted for other factors in the model) are investigated using two logistic regression analyses; one for each measure of lymphatics in synovia. LVD showed a negative association with effusion as the aOR and 95% CI were significantly less than unity. None of the other measures showed a significant relationship with effusion in this study.

lymphatic vessel changes are restricted to this site or representative of diffuse changes across all synovial compartments. Comparing results between surgical and post-mortem tissues is another potential limitation of our study. However, no qualitative differences in podoplanin staining were observed in the current study between samples obtained post mortem and those at joint replacement surgery. Post mortem artefacts would be expected to reduce podoplanin positive vessel densities, leading us to possibly underestimate the differences between OA and post mortem controls. Age and gender were closely matched between disease groups, which reduced the number of possible confounders, although other factors related to OA could not be controlled for. For example, it was not possible in this study to determine whether effusion status explains differences in lymphatic vessel densities between OA and post-mortem controls, as clinical effusions were not assessed in the latter group. Further work is also required to confirm the relationship between effusion and reduced lymphatic density in the OA synovium. We assessed effusion by clinical examination at the time of recruitment. Other measures for quantifying and characterising effusion, for example by ultrasonographic imaging or by biochemical analysis, should be considered to verify and extend our findings. Studies of lymphatic vessel function, including dilation, tissue fluid flow and synovial fluid composition, could further elucidate how decreased lymphatic vessel densities may lead to altered effusion status.

In conclusion, we have found a significant decrease in LVD in OA compared to non-arthritic post mortem synovium, and this was associated with the presence of effusion, but not with synovitis. These findings support the hypothesis that reduction in synovial fluid drainage, due to reduced LVD, may contribute to effusion in OA. Future studies should confirm this association and explore pathophysiological mechanisms underlying these processes.

Author contributions

Study conception: DAW.

Study design: DAW, GJC, PV, DFM, PIM, SA.

Acquisition of data and experimental work: DW, GJC, PV, DFM, SA.

Data analysis and interpretation: GJC, PV, DAW, DFM, PIM, SA.

Drafting and critical review of manuscripts: DAW, PIM, GJC, DFM, PV, DW, SA.

Approval of manuscript: DAW, PIM, DFM, GJC, PV, DW, SA.

Competing interests

The authors declare no potential competing interests.

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Ethical approval

The study was approved by the National Research Ethics Service, reference numbers 05/Q2403/24, 05/Q2403/61, NNHA/420, NNHA/405, NNHA/473 and NN/544. Written informed consent was obtained from all tissue donors or relatives of post mortem cases.

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Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.joca.2012.01.012.

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