TABLE E-1 Neoplasm and Paget's Disease Cases Identified by Histopathological Screening*

Year	Sex	Age *(yr)*	Finding	Time to Clinical Diagnosis *(mo)*
1993	F	66	Suspected myeloma	
1993	M	54	Suspected myeloma	
1994	F	82	Suspected early CLL	
1995	F	73	NHL†	41
1995	F	71	Suspected lymphoma	
1996	M	70	Suspected NHL	
1997	M	57	Suspected lymphoma	
1998	F	65	Suspected lymphoma	
2000	M	62	NHL†	51
2000	M	74	NHL†	1
2001	F	72	NHL†	2
2002	F	84	CLL†	0
2003	F	73	NHL†	0
2003	F	57	CLL†	0
2004	M	46	Suspected CLL	
2004	M	81	Myelodysplastic syndrome†	43
2004	F	80	Suspected CLL‡	
2004	F	76	Suspected low-grade lymphoma	
2004	F	78	Paget's disease	
2006	M	81	NHL†	2
2006	F	71	Paget's disease	

*Long-term follow-up revealed that three patients were diagnosed with malignancy several years after histopathological screening of the femoral head donation revealed findings highly suggestive of a neoplasm. One confirmed malignancy was detected per 770 femoral heads that underwent histopathological screening. CLL = chronic lymphocytic leukemia, and NHL = non-Hodgkin's lymphoma. †Case confirmed with Western Australian Cancer Registry. ‡Cadaveric donor.

TABLE E-2 Criteria for Histopathological Rejection

Initial Histopathological Diagnosis	Criteria
Lymphoid aggregates*	All three hematopoietic lineage precursors identify numerous small lymphoid aggregates, present mostly in an interstitial distribution with one or two lymphoid aggregates in a paratrabecular location (uncertain importance). Lymphoid cells are small and with at most mildly atypical features. Immunohistochemistry shows mixed B-cell and T-cell lymphocyte populations. No features of high-grade lymphoma. No microscopic features of infection. Conclusion: Although aggregates appear reactive in character, it is not possible to completely exclude the possibility of an associated low-grade B-cell lymphoproliferative disorder.
Plasmacytosis	Mild, diffuse infiltrate of plasma cells scattered within the hematopoietic elements. Immunohistochemical staining confirms a mix of kappa and lambda light chain positivity. May be associated with loose fibrosis and edema. No evidence of inflammation or malignancy. Conclusion: Plasmacytosis of unknown importance.
Non-Hodgkin's lymphoma	Marrow space contains a large number of aggregates of small lymphoid cells. Lymphoid cells are small to intermediate in size, with pale-staining, ill-defined cytoplasm and hyperchromatic nuclei that may be cleaved, centrocytic, or centroblastic. Lymphoid cells have a coarsely granular chromatin pattern, thick and slightly irregular nuclear membranes, and small nucleoli. Aggregates are frequently in a paratrabecular location and are widely scattered throughout the marrow space. A reticular pattern is focally increased within these lymphoid aggregates. Immunophenotypic and clonality results required for diagnosis: CD20+, CD79a+, CD5–, CD10–/+, BCL2, BCL6. Conclusion: Confirm with fine needle aspiration, flow cytometry, and polymerase chain reaction (for lambda and kappa light chain restriction detection).
Chronic lymphocytic leukemia	Features of bone marrow infiltration by a low-grade lymphoproliferative disorder. Paratrabecular distribution as well as intertrabecular foci. Small lymphocytes with scant cytoplasm and hyperchromatic, mildly atypical nuclei showing condensed chromatin. Malignant cytoarchitectural features. Immunophenotypic results: CD20+, CD79a+, CD10–, CD5+ (paraffin-embedded sections may be negative due to low sensitivity of detection; flow cytometry is preferable). Conclusion: Confirm with fine needle aspiration, flow cytometry, and polymerase chain reaction (for lambda and kappa light chain restriction detection).
Inflammation (classified as severe osteoarthritis on retrospective review)	Inflammatory cell infiltrate within marrow space. Negative staining for a variety of potential pathogens including bacteria, fungi, and acid-fast bacilli. Conclusion: Although infective etiology is considered unlikely, infection cannot be completely excluded.
Granuloma (classified as severe osteoarthritis on	Well-formed, sarcoid-like, nonnecrotizing granuloma within the hematopoietic marrow. Granulomatous foci are formed by tight,

retrospective review)	concentric collections of epithelioid histiocytes, which are rimmed by small numbers of lymphocytes. No caseation, necrosis, or suppuration. Negative staining for a variety of potential pathogens including bacteria, fungi, and acid-fast bacilli. Conclusion: Most probably related to degenerative fatty changes of no clinical importance, although infection cannot be completely excluded.
Rheumatoid arthritis	Thickened, hyperplastic, and edematous synovium. Infiltration of B cells, CD4+ T cells, plasma cells, and macrophages in synovial stroma. Formation of pannus and osseous ankylosis. Increased vascularity with superficial deposits of hemosiderin. Presence of rice bodies. Increased osteoclastic activity in subchondral bone with associated subchondral cysts and osteoporosis. Conclusion: Confirm with serum, radiologic, and physical examinations and patient history.
Osteochondral metabolic disorder (classified as severe osteoarthritis on retrospective review)	Subarticular bone shows increased osteoblastic activity as well as areas of osteoclastic activity. Between the trabeculae, there is increased fibrous tissue within fatty marrow. Cement lines appear somewhat mosaic-like (possible Paget's disease). Conclusion: Not possible to differentiate between a healing reaction to fracture and metabolic bone disease.
Paget's disease	Bone trabeculae show moderate thickening and disorganization together with an increased number of scalloped cement lines. There is both increased osteoclastic and osteoblastic activity. Marrow demonstrates trilineage hematopoiesis. Cancellous bone with thickened osseous trabeculae with mosaic lamellar pattern and increased osteoclastic and osteoblastic activity, raising the possibility of Paget's disease. Conclusion: Confirm with further biochemical investigations.

*In general, reactive lymphoid collections are usually small in number, well circumscribed, and often contain admixed cellular populations including histiocytes, plasma cells, and mast cells. In benign aggregates, the lymphocytes are typically small in size, with regular monomorphic nuclei with little if any variation in nuclear profile. Follicles with germinal center formation are uncommon, but do not necessarily exclude lymphomatous infiltration. Preferential localization to paratrabecular regions of the marrow space is a feature that particularly raises the possibility of lymphoma. A diagnosis of lymphoma requires the demonstration of clonality. Immunohistochemical studies with pan-B-cell and pan-T-cell markers do not provide information regarding clonality, but the coexistence of lymphocyte populations expressing both T lymphocyte and B lymphocyte markers, although not completely specific, favors a reactive process. Other immunohistochemical markers may be of value; most benign lymphocytic populations within marrow do not express CD10 or CD23. Immunohistochemical demonstration of light chain restriction, using antibodies to kappa and lambda light chains, is generally of limited value in formalin-fixed, paraffin-embedded samples, particularly following decalcification, as the antibodies are usually not sufficiently sensitive to identify immunoglobulin expression.

TABLE E-3 Details of Available Histopathological and Follow-up Investigations for Cases of Malignancy Confirmed with the Western Australian Cancer Registry

Case	Details
1	Fine needle aspiration of an enlarged supraclavicular lymph node was performed. The cytological findings were suggestive of lymphoma, and flow cytometry performed on the aspirate sample demonstrated a clonal population of B cells. Morphological, immunohistochemical, and molecular studies performed on an excisional biopsy of the node established a diagnosis of follicular non-Hodgkin's lymphoma, grade 2. Monoclonal rearrangement of immunoglobulin heavy chain genes detected by polymerase chain reaction studies confirmed the diagnosis of lymphoma.
2	Concurrent bone marrow trephine and excisional biopsy of an enlarged lymph node established a diagnosis of chronic lymphocytic leukemia or small lymphocytic lymphoma. Both the nodal and hematopoietic tissues contained infiltrates of an atypical, monomorphic, B-cell lymphoid population.
3	Bone marrow trephine and aspirate studies were performed. Infiltrates composed of a monotonous population of small, atypical B cells (CD20 and CD79a-positive) were identified. Immunohistochemical studies confirmed the clonal nature of the B-cell infiltrate, with evidence of immunoglobulin (lambda) light chain restriction. CD5, CD10, and cyclin D1 immunohistochemical studies were negative. Further characterization of the low-grade B-cell non-Hodgkin's lymphoma was not possible.
4	DNA was extracted from paraffin blocks made from the retrieved femoral head specimen in an effort to perform polymerase chain reaction studies to identify clonal rearrangements of B-cell and T-cell receptor genes, but insufficient quality DNA was extracted and the polymerase chain reaction studies were not informative. Subsequently, a right cervical lymph node biopsy and bone marrow trephine biopsy were performed. Nodal and marrow involvement by a follicular non-Hodgkin's lymphoma, grade 1, was demonstrated. Immunohistochemical studies demonstrated a CD20+, CD79a+, BCL2+, BCL6+, CD10–, CD23– immunophenotype, and flow cytometry confirmed a clonal B-cell population with kappa light chain restriction.
5	Examination of sections of the femoral head biopsy specimen revealed extensive and monomorphic infiltrates of small, minimally atypical lymphoid cells within the marrow. The atypical infiltrates were located predominantly in paratrabecular areas, with occasional intratrabecular foci. Immunohistochemical studies demonstrated the atypical infiltrate to be CD20-positive, but lacking coexpression of CD5 or CD43. Immunostaining for CD10, BCL6, and cyclin D1 was negative. The findings were felt to be consistent with low-grade B-cell non-Hodgkin's lymphoma, type not further specified.
6*	Microscopic examination of the femoral head biopsy specimen demonstrated paratrabecular aggregates of small to medium-sized, atypical lymphoid cells, which were shown on immunohistochemical staining to have a CD20+, CD79a+, BCL2+, BCL6+, CD10+ immunophenotype. The morphological and immunohistochemical findings were interpreted to be compatible with a follicular non-Hodgkin's lymphoma. Fine needle aspiration of a left

	temporoparietal scalp mass, performed some time later, confirmed the diagnosis of B-cell non-Hodgkin's lymphoma. Flow cytometry demonstrated a CD20+ monoclonal B-cell population with light chain restriction. Subsequent core biopsy of a para-aortic mass demonstrated an atypical lymphoid infiltrate compatible with involvement by the B-cell non-Hodgkin's lymphoma. Flow cytometry was not performed on this sample, and molecular studies were not informative, as insufficient DNA products were obtained for polymerase chain reaction studies.
7*	Microscopic examination of the femoral head biopsy specimen demonstrated an infiltrate of small, atypical lymphoid cells, forming both paratrabecular and intratrabecular aggregates, with morphological and immunohistochemical features consistent with chronic lymphocytic leukemia or small lymphocytic lymphoma. Immunohistochemical studies demonstrated a CD20+, CD79a+, CD5+, CD23+, CD10–, cyclin D1– immunophenotype with possible lambda light chain restriction.
8*	Examination of the femoral head biopsy specimen demonstrated nodular interstitial and occasionally paratrabecular aggregates of small, atypical B lymphocytes strongly suggestive of some form of B-cell lymphoproliferative disorder. Immunophenotypic studies were attempted in sections of the femoral head biopsy specimen but were not informative. Immunohistochemical stains for CD20, CD3, CD5, CD10, and kappa and lambda light chain expression were technically suboptimal, and could not be accurately evaluated, possibly because of poor antigen preservation related to tissue processing, including decalcification.

*No further information regarding subsequent investigations was available, although a definitive diagnosis of lymphoma was recorded in the Western Australian Cancer Registry database.