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ORIGINAL ARTICLE

Characterization of bone marrow CD4 to CD8 ratios and lymphocyte composition in adults by image analysis

Brian D. Adkins[1] · Nicholas R. Jaeger[1] · Rachel M. Whitehair[1] · Patcharin Pramoonjago[1] · Nadine S. Aguilera[1]

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Abstract
Bone marrow (BM) lymphocyte subsets are evaluated by flow cytometry or immunohistochemistry for diagnostic purposes; however, CD4:CD8 T lymphocyte ratios are often erroneously interpreted using peripheral blood ranges. There are few and no recent studies describing the composition of lymphocytes within the marrow space, or normal reference ranges. Lymphocyte subsets in cytopenic patients and hospital autopsy BM specimens were evaluated to better characterize CD4:CD8 ratios. Ten patients with a history of cytopenia were identified from 2017 to 2021. Clinical history, cytogenetic testing, and results of a next generation sequencing panel were reviewed to rule out hematolymphoid disease. Thirty-five decedents who underwent a hospital autopsy from 2018 to 2019 were identified. History of hematolymphoid disease was ruled out by chart review. Immunohistochemical staining for CD3, CD20, CD4, and CD8 was evaluated with digital image analysis. Findings were compared to peripheral blood flow cytometry in a group of 20 living patients. BM CD4:CD8 ratios by image analysis were significantly lower than peripheral blood, mean in cytopenic patients 0.37:1 and mean in decedents 0.51:1 versus 2.6:1 ($p = < .001$ in both groups). BM CD4:CD8 ratios were significantly lower ($p = 0.04$) than ratios found using flow cytometry on the same specimen, suggesting hemodilution. There was no significant difference in CD4:CD8 ratios when comparing living patients and decedents' marrows ($p = > 0.99$). Lymphoid aggregates were encountered with increasing frequency in older individuals. These findings aid in the evaluation of BM lymphocyte subsets and distribution both in living patients and autopsy evaluation. We also present a practical approach to image analysis.

Keywords CD4 to CD8 ratio · T lymphocytes · Bone marrow · Image analysis · Autopsy

Introduction

Evaluation of bone marrow by flow cytometry and/or immunohistochemistry (IHC) is performed routinely in hematopathology for diagnostic and clinical purposes, and T lymphocyte percentages are often interpreted using peripheral blood reference ranges, which may not be appropriate as CD8 positive T-cells predominate in the bone marrow. Unlike peripheral blood where CD4:CD8 ratios are conventionally around 1–3:1, higher numbers of cytotoxic T-cells in bone marrow lead to a reduced or inverted CD4:CD8 T-cell ratio with reported averages ranging from 0.55 to 1.11 [1–6]. The finding of a low CD4:CD8 ratio in bone marrow by flow cytometry may be misinterpreted as abnormal when compared to peripheral blood, when in fact in the bone marrow specimen, it is a characteristic normal finding.

Most knowledge of normal lymphocyte distribution in the bone marrow is based on studies which were completed decades ago with either limited flow cytometry or more archaic techniques such as erythrocyte rosetting in small cohorts [1–5, 7, 8]. Bone marrow biopsy is an invasive procedure, and aspirate materials are not available as discarded specimens for determining reference ranges, unlike leftover peripheral blood specimens which can be easily obtained. As such, most 'normal' bone marrow biopsies are obtained for cytopenia evaluation and are performed infrequently. Autopsy decedents represent a group of general hospitalized patients with bone marrow materials, a potential repository of available specimens for evaluation.

Bone marrow samples are commonly obtained at autopsy. Evaluation of bone marrow morphology is routine in hospital autopsy practice, though it is generally performed by surgical pathologists using H&E morphology. Histologic

✉ Nadine S. Aguilera
NA2D@hscmail.mcc.virginia.edu

[1] University of Virginia Health System, 1215 Lee St. Box, Charlottesville, VA 22908, USA

evaluation of autopsy marrow may demonstrate previously undiagnosed findings such as metastatic and hematolymphoid neoplasms, or more commonly benign lymphoid aggregates, which occur at greater frequency in older decedents [9]. Few studies are available evaluating T and B cell lymphocyte subsets of autopsy bone marrow, though a prior study comparing bone marrow findings in sudden death autopsies against autopsies performed on hematopoietic stem cell transplant patients demonstrated lymphocytes consisting predominantly of T-cells with an average CD4:CD8 ratio of in 1.03 in 9 non-transplant patients and 0.56 in 13 transplant patients [4].

Normal CD4 to CD8 ratios in bone marrow are uncharacterized in the recent medical literature. As such, we sought to evaluate bone marrow lymphocyte populations using modern IHC staining and image analysis in cytopenic patients without underlying hematolymphoid diseases and hospital autopsy decedents. These results were then compared with control flow cytometric findings in the peripheral blood of living patients without lymphoproliferative disease.

Materials and methods

Study populations

Living patient group

This study was approved by our institutional review board (IRB) protocol #13,310. The laboratory information system (Epic Beaker, Epic Systems Corporation, Verona, WI, USA) was queried from 2017 to 2021. Normocellular bone marrow biopsies with the indication of pancytopenia or anemia were queried. Patients with HIV, known immunodeficiency, or history of hematologic malignancy (leukemia, lymphoma, myelodysplastic syndrome, or myeloproliferative disorder) were excluded from this study; autoimmune disease was not excluded based on chart review. The search was expanded to include one hypercellular marrow, due to a low number of cases. Charts were queried for history of hematolymphoid disease. Likewise, molecular testing via the TruSight Myeloid Sequencing Panel (Illumina San Diego, CA, USA) and cytogenetic results in the form of karyotyping and fluorescence in-situ hybridization were reviewed, with abnormal clonal findings being used as exclusion criteria. Corresponding, concurrent flow cytometry results of bone marrow specimens which were evaluated for CD4 and CD8 were also queried.

Autopsy group

Autopsy records were queried within the laboratory information system 2018–2019. Decedents' autopsy reports were checked to ensure consent for medical research. Autopsy reports and clinical history were reviewed. Decedents with a history of HIV, known immunodeficiency, or hematologic malignancy (leukemia, lymphoma, myelodysplastic syndrome, or myeloproliferative disorder) were excluded from this study based on chart review. Patients who died suddenly due to thromboembolic disease (myocardial infarction, cerebrovascular accident, or pulmonary embolism), hypoxic ischemic injury, hemorrhage, or trauma or those who expired due to acute infectious or inflammatory conditions such as sepsis, bronchopneumonia, diffuse alveolar damage, or acute pneumonia were selected.

Peripheral control group selection

Peripheral blood flow cytometry from patients with no known history of lymphoproliferative disorder was used as a control to establish a normal blood CD4 to CD8 ratio and as a comparison for CD4 to CD8 ratio. Flow cytometry was performed to "rule out" a variety of processes in these patients. Flow cytometry reports were reviewed to determine the average CD4:CD8 ratio in peripheral blood.

Bone marrow clot preparation

Living patient bone marrow clot materials were utilized. Clot material is obtained routinely during bone marrow biopsy procedure by allowing aspirate fluid remaining after slides are made to clot and then processed without the use of decalcification.

Autopsy bone marrow was obtained from rib sections stored in formalin from 20 to 413 days. Marrow was extracted by compressing sections of rib with angled forceps similarly to the method described by Schloegl et al. [10]. Extracted marrow was placed in mesh bags and submitted for processing and IHC evaluation.

CD3 and CD20 immunohistochemistry

IHC was performed on a robotic platform (Ventana discover Ultra Staining Module, Ventana Co., Tucson, AZ, USA). Tissue Sects. (4 µm) were deparaffinized using EZ Prep solution (Ventana). A heat-induced antigen retrieval protocol set for 64 min was carried out using Cell Conditioner 1 (Ventana). Endogenous peroxidases were blocked with peroxidase inhibitor (CM1) for 8 min before incubating the section with CD3 antibody (Dako, Cat#A0452) and CD20 (Leica Biosystems, Cat# NCL-CD20-L26) at 1:300 and 1:100 dilutions, respectively, for 60 min at room temperature. Antigen–antibody complex was then detected using DISCOVERY. OmniMap anti-rabbit multimer and mouse RUO detection systems, respectively, and the complexes were visualized by DISCOVERY ChromoMap DAB

Kit (Ventana Co.). All the slides were counterstained with hematoxylin subsequently; they were dehydrated, cleared and mounted for the assessment.

CD4 and CD8 immunohistochemistry

Four micron histologic sections were cut, placed on charged glass slides (Superfrost Plus, Fisher Scientific, Pittsburgh, PA, USA). Slides were then deparaffinized and antigen retrieval was performed in PT Link instrument (Dako, Glostrup, Denmark) in high pH antigen retrieval solution at 97° C for 20 min. IHC was performed on a robotic platform (Autostainer, Dako). Endogenous peroxidases were blocked using Peroxidase and Alkaline Phosphatase Blocking Reagent (Dako). Polyclonal rabbit antibody to CD4 (Leica Biosystems, Cat# NCL-L-CD4- 358) and CD8 (Dako, Cat#M7103) were diluted at 1:120 and 1:300 dilutions, respectively, and applied at ambient temperature for 60 min. Antibody binding was visualized by incubation with EnvisionTM Dual Link (Dako) followed by incubation with 3,3'-diaminobenzidine tetrahydrochloride (DAB +). All the slides were counterstained with hematoxylin subsequently; they were dehydrated, cleared and mounted for the assessment. Histiocytes and monocytes were used as an internal control for CD4 staining.

Staining adequacy and visual analysis

Staining and distribution of cells CD3, CD20, CD4, and CD8 IHC were reviewed by two pathologists. A consensus estimate of CD3 and CD20 percent cellularity was determined. Lymphoid aggregates were noted and characterized as mixed, T-cell predominant, or B-cell predominant.

Image analysis

The entire slide was reviewed, and representative 200 × images were obtained using two microscopes (Leica Microsystems, Buffalo Grove, IL, USA) equipped with microscope cameras (Infinity 1, Ottawa, Ontario, Canada) and included camera software (Infinity Analyze, Ottawa, Ontario, Canada). Image analysis was performed using ImageJ (National Institutes of Health, Bethesda, MD, USA). Cells of interest were circled and the image area in pixels was measured. Images were then converted to 8-bit grayscale and the threshold was manually adjusted to determine the percent cellularity. This percentage was then multiplied by the total area of the image in pixels to determine the area of marrow. The percentage of cells of interest was then determined by dividing the area of cells of interest by the area of marrow in pixels.

Statistics

Statistics were performed using PRISM 8 (GraphPad Software, San Diego, CA, USA). Continuous variables were compared using a Mann–Whitney U test and categorical variables were compared using a Fisher's exact test. Paired variables were evaluated using a Wilcoxon test. A p value of < 0.05 was considered significant. The multiple testing hypothesis was accounted for by using Bonferroni adjusted p-values.

Results

Living patients

Patient demographics are as follows: 6 female and 4 male with an average age of 60.10 years range 39–90). Of the 10 patients evaluated, half were biopsied for anemia and the other half were biopsied for pancytopenia. Underlying causes of cytopenias were identified clinically in half of the patients and included: cirrhosis 2/10 (20%), kidney failure 1/10 (10%), iron deficiency 1/10 (10%), and urothelial carcinoma 1/10 (10%). None of the patients had definitively pathologic molecular or cytogenetic findings, though two patients had variants of undetermined significance with NGS testing (Table 1).

IHC staining was adequate in all cases. The average CD4:CD8 ratio was 0.37:1. Lymphoid aggregates were present in half of the cases reviewed though additional staining had only been performed in one case clinically, and showed mix kappa and lambda expression by in-situ hybridization. Other findings are reviewed in Table 1. The CD4:CD8 ratio was higher in marrows with lymphoid aggregates 0.5 vs 0.24 ($p = 0.4$).

The peripheral blood control group contained 20 patients with negative peripheral blood flow cytometry (no evidence of monotypic B-cells or atypical T-cell populations). The average CD4:CD8 ratio was 2.6:1 (range 0.33–6.1). The CD4:CD8 ratio in peripheral blood was higher and significantly different from the ratio demonstrated in cytopenic bone marrows evaluated ($p = < 0.001$).

Corresponding bone marrow flow cytometry was compared with results obtained by image analysis. The CD4:CD8 ratio by flow was significantly higher 2.06:1 versus 0.37:1 ($p = 0.04$), and the total lymphocyte count was lower 8% versus 20.98% ($p = 0.12$).

Decedents

Autopsy decedent demographics are as follows: 16 female and 19 male decedents with an average age 58.90 years (range 27–89). Of the 35 decedents evaluated, 23/35 (66%)

Table 1 Characterization of lymphocytes in patients with anemia/pancytopenia using digital image analysis

Case	Age	Sex	CD3	CD20	CD3+CD20	Total by FC	Lymphoid aggregates	Aggregate composition	CD4	CD8	CD4:CD8 ratio	CD4:CD8 ratio by FC	NGS mutation(s)	Cytogenetic testing
1	49	Male	12.84%	6.85%	19.69%	5%			3.15%	4.49%	0.70	2.47	None	Negative
2	70	Female	4.88%	5.60%	10.48%	3%	Present	Mixed	2.32%	4.79%	0.48	1.74	None	Negative
3	62	Male	8.33%	2.37%	10.69%	5%			5.40%	8.18%	0.66	3.00	None	Negative
4	48	Female	11.04%	3.65%	14.69%	6%	Present	TCP	1.65%	18.75%	0.09	1.06	None	Negative
5	64	Female	39.59%	2.21%	41.80%	8%			4.36%	12.73%	0.34	1.86	-	Negative
6	62	Female	14.94%	4.78%	19.71%	6%	Present	Mixed	1.31%	6.53%	0.20	2.8	None	Negative
7	39	Female	10.69%	7.22%	17.91%	24%	Present	TCP and BCP	3.96%	19.42%	0.20	3.94	None	Negative
8	49	Male	15.34%	0.69%	16.03%	6%			6.87%	12.79%	0.54	0.65	DNMT3A	Negative
9	68	Male	15.68%	6.81%	22.49%	6%	Present	BCP	1.25%	7.11%	0.18	1.54	-	Negative
10	90	Female	33.95%	2.34%	36.29%	10%			4.60%	17.66%	0.26	1.54	BCOR TET2	-
Mean/Total	60.10	6 Female 4 Male	16.73%	4.25%	20.98%	8%	50% (5/10)		3.49%	11.25%	0.37	2.06		

CD3, CD20, CD4, and CD8 subsets are displayed as percentage cells of total bone marrow cellularity. *TCP*, T-cell predominant; *BCP*, B-cell predominant; *FC*, flow cytometry

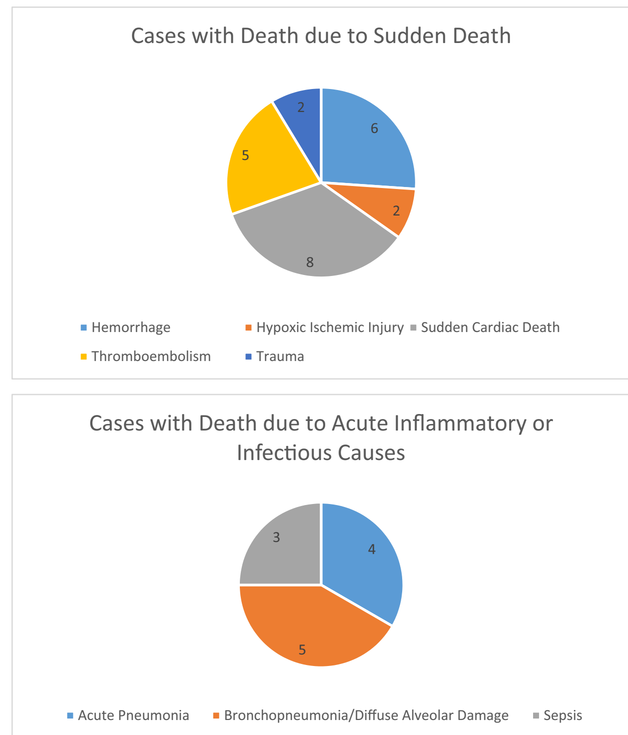

Fig. 1 Cause of death in decedents with sudden death and those who expired due to acute inflammatory or infectious causes

died suddenly while 12/35 (34%) expired due to acute infectious or inflammatory processes (Fig. 1). Past medical history was available in all patients. The most common past medical histories included: hypertension (72.22% of cases 26/35), hyperlipidemia (63.89% of cases, 23/35), and diabetes mellitus, unspecified (27.78% of cases, 10/35). Autoimmune disease was identified in 3/35 patients (8.57%), and disease histories included: rheumatoid arthritis, Hashimoto's thyroiditis, and Graves' disease. Cancer histories among patients included 4 patients with carcinoma (decedent 5, colon and urothelial; decedent 7, breast; decedent 25, squamous cell carcinoma and basal cell carcinoma; and decedent 34, endometrioid adenocarcinoma) and 2 patients with history of melanoma (decedent 18 and decedent 29).

IHC staining for CD3 and CD8 was adequate in all cases and is summarized in Table 2 (example Fig. 2). CD4 staining failed in 3 of 35 cases (8.57%). Time to prosection was on average 1 day 6 h and 49 min and ranged from 3 h and 3 min to 5 days 4 h and 4 min. Specimens were stored in formalin prior to processing for an average 194 days with a range of 20 days to 413 days. CD4 staining failed in three cases which were stored in formalin for more than 150 days (case 22, 407 days, and case 34, 288 days, and case 10, 185 days) but did not have prolonged time prior to prosection (case 22, 9 h 1 min, and case 34, 1 day 21 h 26 min and [case 10] 1 day 0 h 35 min). CD20 staining failed in one case (2.86%)

Table 2 Characterization of lymphocytes in autopsy patients using digital image analysis

Case	Age	Sex	CD3	CD20	CD3 + CD20	Lymphoid aggregates	Aggregate composition	CD4	CD8	CD4:CD8
1	65	Male	9.68%	1.48%	11.16%			4.62%	5.16%	0.89
2	52	Male	12.55%	5.50%	18.05%	Present	Mixed	0.77%	5.43%	0.14
3	73	Male	4.86%	0.27%	5.14%			0.84%	2.71%	0.31
4	35	Male	8.69%	2.87%	11.57%			1.27%	1.73%	0.73
5	71	Male	9.85%	1.80%	11.65%			2.11%	9.82%	0.22
6	27	Female	13.59%		13.59%			1.50%	9.45%	0.16
7	78	Female	13.54%	2.44%	15.98%	Present	BCP	1.16%	6.16%	0.19
8	46	Male	10.81%	1.81%	12.62%			3.57%	9.74%	0.37
9	67	Female	6.67%	2.78%	9.45%	Present	Mixed	2.93%	4.35%	0.67
10	33	Female	10.00%	1.62%				-	3.02%	-
11	72	Female	11.05%	2.39%	13.44%			1.56%	4.52%	0.35
12	69	Male	8.84%	1.49%	10.33%	Present	BCP	3.20%	2.90%	1.11
13	83	Female	15.07%	5.17%	20.24%			5.59%	2.58%	2.17
14	74	Male	9.81%	0.90%	10.70%			1.69%	2.04%	0.83
15	69	Female	10.16%	3.78%	13.94%	Present	TCP	1.05%	2.58%	0.41
16	69	Female	21.92%	1.18%	23.10%			0.60%	12.19%	0.05
17	56	Male	3.85%	1.10%	4.95%	Present	Mixed	2.18%	2.86%	0.76
18	44	Female	10.69%	0.59%	11.27%	Present	TCP	1.37%	3.69%	0.37
19	89	Female	10.55%	0.56%	11.11%			3.21%	4.53%	0.71
20	37	Male	15.51%	2.63%	18.14%			4.28%	10.30%	0.42
21	72	Female	12.17%	0.40%	12.57%	Present	TCP	1.90%	3.08%	0.62
22	50	Male	7.95%	0.31%	8.26%			-	2.44%	-
23	60	Male	11.27%	0.72%	12.00%			0.42%	1.08%	0.39
24	52	Female	8.77%	3.92%	12.69%	Present	BCP	1.74%	4.16%	0.42
25	78	Male	9.84%	2.87%	12.70%			1.28%	6.82%	0.19
26	39	Male	6.76%	1.33%	8.09%			0.40%	1.75%	0.23
27	37	Male	6.34%	5.02%	11.36%			1.44%	2.23%	0.65
28	60	Female	5.57%	0.72%	6.28%			1.23%	2.12%	0.58
29	59	Male	9.17%	1.49%	10.66%	Present	Mixed	3.09%	4.79%	0.64
30	30	Male	11.80%	1.11%	12.91%			0.18%	4.33%	0.04
31	65	Male	10.13%	2.16%	12.28%			1.88%	3.01%	0.62
32	68	Male	6.42%	2.52%	8.94%			0.39%	3.44%	0.11
33	52	Female	7.29%	0.31%	7.60%			1.30%	2.41%	0.54
34	72	Female	13.23%	1.26%	14.49%	Present	BCP	-	6.43%	-
35	48	Female	6.74%	0.74%	7.47%			0.74%	1.76%	0.42
Overall	58.63	16 Female 19 Male	10.03%	1.92%	11.85%	31.43% (11/35)		1.87%	4.39%	0.51

CD3, CD20, CD4, and CD8 subsets are displayed as percentage cells of total bone marrow cellularity. Cases in bold expired due to acute inflammatory or infectious causes. *TCP*, T-Cell predominant; *BCP*, B-cell predominant

without prolonged storage or time to prosection (stored in formalin 49 days with 12 h 15 min to prosection).

The average CD4:CD8 ratio was 0.51:1 (range 0.04–2.17:1). The CD4:CD8 ratio in peripheral blood was higher and significantly different from the ratio demonstrated in autopsy bone marrows evaluated ($p = < 0.001$). There was no significant difference when comparing ratios in living patients (iliac samples) and deceased patients (rib squeeze samples) ($p = > 0.99$). The average percentage of lymphocytes of the total bone marrow cellularity was 11.85% (range 4.95–23.09%). There was no significant difference between the sudden death decedents or those who expired due to acute infectious or inflammatory conditions in the number of total lymphocytes, B-lymphocytes, T-lymphocytes, CD4 positive T-lymphocytes, CD8 positive T-lymphocytes, or CD4 to CD8 ratios ($p = 0.77$—> 0.99) (Table 2). The CD4:CD8 ratio was higher in marrows with lymphoid aggregates 0.53 vs 0.50 ($p = > 0.99$).

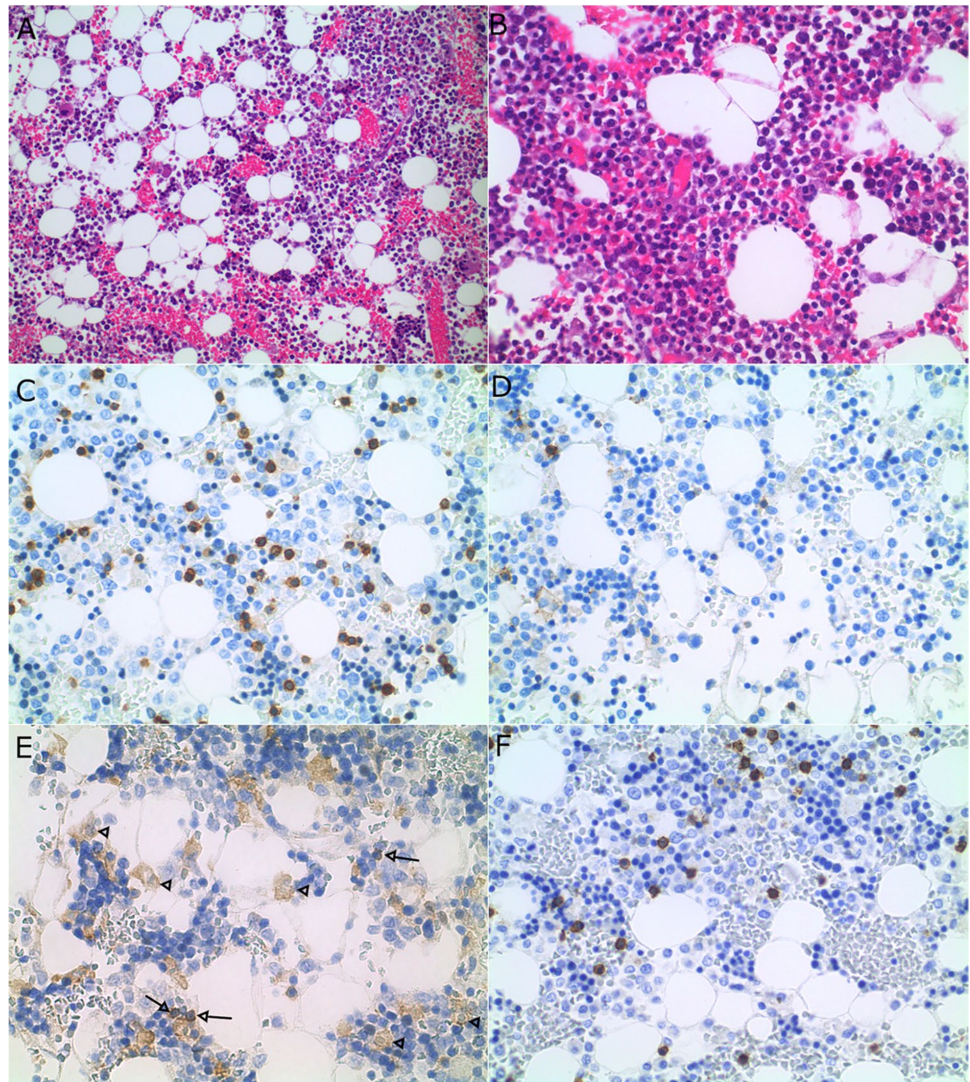

Fig. 2 All photomicrographs were obtained from representative case number 29. This decedent expired due to bilateral acute bronchopneumonia. The time to prosection was 1 day 19 h and 14 min. The tissue was stored in formalin for 150 days prior to processing. **A** Bone marrow H&E at 200× magnification showing minimal autolytic changes. **B** High power bone marrow image at 400×. **C** CD3 demonstrating T lymphocytes. **D** CD20 staining relatively fewer B lymphocytes. **E** CD4 staining small lymphocytes (arrow with tail) and background histiocytes (arrow without tail). **F** CD8 staining proportionally increased cytotoxic T-cells

Discussion

There was a significant difference in CD4:CD8 ratios in bone marrow compared to peripheral blood in living patients ($p = 0. < 001$). This suggests that the bone marrow space is relatively enriched for CD8 positive T-cells. There were a limited number of normocellular bone marrow specimens in living patients with cytopenias without underlying hematolymphoid processes and these numbers were supplemented with autopsy materials. Rib squeeze specimens demonstrated excellent immunoreactivity in spite of variable time to prosection and extended formalin-fixation prior to processing; therefore, autopsy marrow specimens proved to be a viable option to assess marrow lymphocyte subsets by IHC. Our findings are supported by early investigations that also show CD4:CD8 ratios are reduced in the marrow compared to the peripheral blood [1–5].

Lymphoid aggregates were encountered in 50% of living patients and 31% of autopsy bone marrows. As none of the patients had a history of hematolymphoid disease, and most of the aggregates were found in older decedents, these are likely non-neoplastic or so called "physiologic" aggregates [9, 11]. The majority of aggregates were mixed or T-cell predominant, though both groups contained some cases with B-cell predominant aggregates. No further staining was performed by our group. Due to the collection method, it was not possible to determine the architectural distribution of the aggregates, which can be informative as paratrabecular aggregates are more often pathologic. Johnston et al. reviewed lymphoid aggregates in bone marrow to determine findings that are suggestive of malignancy which include infiltrating edges, paratrabecular location, large size, cytologic atypia, or B-cell predominance [12]. Lymphoid aggregates are commonly encountered at autopsy, especially with increasing age, though based on CD3 and CD20 staining, most are likely physiologic in nature. Careful morphologic and architectural evaluation should help guide utilization of IHC.

Image analysis was utilized, as a percentage area of cells can be calculated, limiting the subjectivity of IHC quantification. Manual image analysis was chosen as it is easily performed on still photomicrographs and does not require expensive whole slide imaging equipment. Likewise, manual image analysis proved to be useful when evaluating CD4 staining, as monocytes must be differentiated from T-cells by morphologic differences and staining alone as detected by automated techniques could lead to erroneously high CD4 percentages. This technique did however result in higher lymphocyte percentages than in flow cytometry specimens, though this should not impact CD4:CD8 ratio findings. Utilization of ImageJ, a publicly available software, designed to evaluate still images, could prove useful in routine clinical situations where determination of percentage positivity is significant such as number of CD34 positive blasts in myeloid disease, number of CD138 positive plasma cells in multiple myeloma staging marrows, or percent PD-L1 expression in non-small cell lung cancer.

Bone marrow aspiration specimens often have significant peripheral blood contamination, which may lead to artificially increased CD4:CD8 ratios by flow cytometric evaluation. Our living patient group had corresponding CD4:CD8 ratios by flow cytometry which were significantly higher and may suggest peripheral blood contamination. This may be the case in the reported ratio of 1.11 by Clark and Normansell which was determined using bone marrow aspirate specimens [1]. They concede in their discussion that they could not definitively determine whether lymphocytes were derived from peripheral blood or bone marrow, though cells can be accurately localized on histologic sections [1]. Hemodilution has a significant impact on the proportions of cells characterized by flow cytometry, with an early study showing variability and high levels of dilution in aspirate specimens, ranging from 6 to 93% [13]. Additional studies have shown that the number of draws and volume of aspirate material affect the amount of hemodilution and that even the style of needle may be impactful [14–16]. Increased lymphocytes have been shown to be associated with increased peripheral blood contamination in bone marrow aspirates, which is especially important as lymphocytes compose only a small portion of the marrow cellularity and even an incremental increase may skew findings [17]. As the amount of hemodilution was not determined in prior flow cytometry studies, IHC on permanent sections may represent a superior method of bone marrow quantification [1–3].

There was some concern about autolysis in decedent samples, as many autopsy specimens undergo significant autolytic changes in the post-mortem period, though histologic examination demonstrated adequate specimens (Fig. 2). A recent forensic study showed decreased autolysis in autopsy bone marrow specimens compared to other tissues, with the most marked autolytic changes occurring in erythroid cells as opposed to myeloid, megakaryocytic, or lymphoid cells [18]. Likewise, IHC was successful in most of our cases. To that end, the rate at which specific antigens degrade in formalin is unclear, though it appears that most remain intact, even with prolonged formalin storage time.

The finding of a reduced CD4:CD8 ratio in bone marrow is impactful as this may be unanticipated if pathologists are not familiar with expected normal ranges. An inverted CD4 to CD8 ratio may be misinterpreted as abnormal in the bone marrow leading to inaccurate reporting or additional testing such as larger flow cytometry panels or unnecessary IHC stains. Bone marrow evaluation at autopsy yielded some anticipated findings such as lymphoid aggregates and low percentage of lymphocytes within the marrow composed predominantly of T-cells. The manual CD4 to CD8 ratio estimate in the autopsy group was similar to that of the image analysis; therefore, manual assessment of the CD4 to CD8 ratio may be appropriate in clinical pathology settings where flow cytometry or image analysis is not available. To avoid over interpretation, practicing hematopathologists and pathologists performing autopsies should be aware of the low CD4 to CD8 ratio and familiar with physiologic lymphoid aggregates.

Declarations

Conflict of interest The authors declare no competing interests.

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