Original Article

The Diagnostic Value of Kappa/Lambda Ratios Determined by Immunohistochemistry in the Differentiation of Plasma Cell Myeloma from Reactive Plasmacytosis

Yunes Shafieno[1], Babak Izadi[1], Sedigheh Khazaei[2], Maryam Foroughikia[3], Sara Hookari[4], Sima Nazemi[1], Mitra Tarlan[2]

[1]Molecular Pathology Research Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran, [2]Clinical Research Development Center, Imam Reza Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran, [3]Department of Infection Disease, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran, [4]Department of Biostatistics, Substance Abuse Prevention Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Context: Plasma cell myeloma (PCM) is the second-most common B-cell malignancy in western countries. In this disease, the bone marrow is infiltrated by monoclonal plasma cells, which may secrete immunoglobulin light chains. In this line, reactive plasma cells may infiltrate into the bone marrow that can lead to misdiagnosis of the disease. **Aims:** The purpose of this study was to determine monoclonality using immunohistochemistry (IHC) for kappa and lambda light chains in bone marrow biopsy (BMB) specimens. These findings may be useful in the differentiation of PCM from reactive plasmacytosis. **Materials and Methods:** In this study, BMB paraffin blocks from 90 patients including 45 cases of PCM and 45 patients with reactive plasmacytosis were collected at the pathology center of Imam Reza Hospital from 2006 to 2014. IHC staining for CD138 and Kappa-Lambda light chains were performed. Relative frequencies of Kappa to Lambda light chain positive cells were calculated. **Statistical Analysis Used:** The results were analyzed with spss16 and Rx64 3.1.2 software. **Results:** Strong Kappa staining was found in 37 of 45 PCM cases (82.2%), with the ratio of Kappa/Lambda>3 and eight other cases (17.8%) expressed Kappa/Lambda<0.48. In cases with reactive plasmacytosis, K/L ratio was $0.48 \leq \times \leq 3$. Sensitivity and specificity for the diagnosis of PCM versus reactive plasmacytosis were 100% and 97.8%, respectively. **Conclusions:** Results indicated that immunohistochemical staining of CD138, kappa, and lambda light chains could be considered as an assured and reliable assay for the diagnosis of monoclonal plasma cells and differentiating it from other hematological malignancies.

Keywords: *CD138, immunoglobulin light chains, immunohistochemistry, plasma cell myeloma, plasmacytosis*

Introduction

Plasma cell dyscrasia is a general term for a plethora of diseases characterized by the dispensable proliferation of immunoglobulin generating cells and includes plasma cells myeloma (PCM), plasmacytoma and immunoglobulin aggressive diseases.[1-3] Plasma cell myeloma (PCM) is a neoplastic proliferation of plasma cells with excessive secretion of monoclonal immunoglobulins in serum and urine. PCM is classified into several clinical variants, including nonsecretory myeloma, asymmetric myeloma, plasma cell leukemia, and osteosclerotic myeloma.[4] PCM is the second-most common hematologic malignancy of B cells with 2% of cancer-related death that encompasses about 15% of blood malignancies.

The prevalence of PCM among young people is extremely low and is usually detected in patients with a median age of 70-old-year at the time of diagnosis. Besides, the male population indicates a higher incidence of PCM than the female group with a ratio of 1.4:1.[5] According to a report in 2012, 10,710 mortalities were found among 21,700 new cases of PCM diagnosed in the United States. Although there is a lack of geographical spreading about PCM, the incidence of PCM goes up in European countries such as the United Kingdom, Switzerland, Italy, and down in developing countries.[5,6]

Among common risk factors, toxic and genetic damages were found to play the pivotal roles favoring the pathogenesis of PCM.[5] The general symptoms accompanied by PCM include anemia, osteolytic lesions, hypercalcemia, renal dysfunction, and systemic infections, in which bone pain is the most common appearance in the patients.[7]

How to cite this article: Shafieno Y, Izadi B, Khazaei S, Foroughikia M, Hookari S, Nazemi S, *et al*. The diagnostic value of kappa/lambda ratios determined by immunohistochemistry in the differentiation of plasma cell myeloma from reactive plasmacytosis. Clin Cancer Investig J 2019;8:177-81.

Address for correspondence:
Dr. Babak Izadi,
Molecular Pathology Research Center, Imam Reza Hospital, Zakaria Razi Bol, Kermanshah, Iran.
E-mail: bizadi@hotmail.com

Access this article online

Website: www.ccij-online.org

DOI: 10.4103/ccij.ccij_65_19

Quick Response Code:

The diagnosis of PCM is based on the World Health Organization (WHO) criteria considering the clinicopathological and radiological features of plasma cells.[8] In this line, the main principles are the extents of immunoglobulins and paraproteins (more than 3.5 g/dl IgG or more than 2 g/dl of IgA and more than 1 g/dl of Bence Jones protein) in 30% of plasma cells in bone marrow and plasmacytoma. Furthermore, accessorial factors, including the reduced level of normal monoclonal immunoglobulin in serum and osteolytic disorders in 10%–29% of plasma cells in the bone marrow.[8]

While morphological and histological parameters are valuable to evaluate PCM, it is essential to distinguish reactive plasmacytosis from neoplastic form (PCM) by determining the expression of Ig heavy and light chains.[9] Observing monoclonal light chain disorder was considered as a prominent feature of the monoclonal gammopathies. The measurement of free light chains (κ and λ) appears to be a fundamental approach in the diagnosis of monoclonality. On the other hand, CD138 as a proteoglycan containing heparin sulfate seems to be involved in cell adhesion, regulation of cellular growth, and apoptosis,[10,11] which is exclusively expressed in plasma cells among other cells in the bone marrow. Therefore, CD138 is used as the diagnostic marker to isolate and identify plasma cells.[12,13]

Taking everything into consideration, the purpose of this study was to investigate the expression of CD138, kappa, and lambda light chains and their ratios through immunohistochemistry (IHC) to find the monoclonality of PCM.

Materials and Methods

Monoclonal mouse Anti-Human CD138, Polyclonal Rabbit Anti-Human Kappa light chain, Polyclonal Rabbit Anti-Human Lambda light chain, Biotin Blocking System, Liquid 3,3′-diaminobenzidine tetrahydrochloride (DAB)+Substrate Chromogen System, EnVision and Dual Link System, and Target Retrieval Solution were provided from DAKO (Denmark). Hematoxylin was purchased from Panreac (Spain). Hydrogen peroxide, methyl alcohol, Entelan glue, and ethyl alcohol 99.6% were supplied from Merk (Germany). Pepsin was provided from Sigma (Germany). Xylene and ethyl alcohol 96%–70% were purchased from Shimi-nab (Iran).

Patient samples

The paraffin blocks of bone marrow tissues with a diagnosis of PCM and reactive plasmacytosis (according to the WHO criteria) from 2006 to 2014 were retrieved from archives. According to statistical analyses, the number of 90 samples (45 PCM and 45 reactive plasmacytosis) was chosen to evaluate. Ethics committee approval was waived because no patients were identified in the study.

Hematoxylin and eosin staining

Paraffin-embedded tissues were provided as 4 μm sections and stained with hematoxylin and eosin method. In this method, glass slides containing tissue sections incubated at 70°C for 2 h. Then, slides rinsed into several jars filled with xylene, graded series of ethanol solutions, hematoxylin, lithium carbonate, and eosin. Two pathologists independently evaluated stained sections.

Immunohistochemistry

Immunohistological staining was performed on formalin-fixed paraffin-embedded tissue sections using antibodies against CD138, kappa light chain, and lambda light chain. For this aim, 4 μm tissue sections were deparaffinized at 37°C for 2 h and Xylene for 24 h. Then, slides rehydrated in a graded series of ethanol solutions and PBS (phosphate-buffered saline) for about 12 min. To retrieve antigens slides immersed in the jar containing Tris buffer (pH = 9) and heated in a water bath at 95°C for 20 min followed by washing in PBS solution. To quench the intracellular activity of peroxidases, slides were immersed in a solution of 3% hydrogen peroxide in methanol for 10 min, washed with PBS and placed in jars containing iodine solution for 5 min. Then, biotin was added to increase the specificity of staining. After washing with PBS, slides were incubated by primary and secondary antibodies for 45°C and 30°C, respectively in a humid and dark place at room temperature. The slides were washed in PBS and stained with the substrate–chromogen solution known as DAB for 5 min. The counterstaining was performed with hematoxylin for 30 s and washed in water. The stained slides immersed in a graded series of ethanol and then, xylene to transparency and dehydration of tissues. Subsequently, slides mounted to study under a microscope. Negative controls were exposed to antibody diluent replacing primary antibody.

Statistical analyses

Data were analyzed using SPSS16 software ((SPSS Inc., Chicago, IL, USA)). The study of kappa/lambda ratios was performed using Rx64 3.11 software. The kappa/Lambda ration was defined as the ratio of kappa-positive cell numbers to lambda positive cell numbers in 5 fields (×40) and analyzed using R statistical software (http://www.rproject.org). In this method, u and l were considered as lower and upper cutoff levels. The frequency table was created at the independent movement of upper cutoff from u = 1 to u = 10 with the pitches of 0.1 unit as well as lower cut-off from l = 0 to l = 0.99 with the pitches of the 0.01 unit by 9191 frequency tables [Table 1a and b]. Then, the specificity, sensitivity, and error rates were measured based on equations. The final response was chosen according to the frequency with the minimum error rates (maximum sensitivity and specificity).

Results

CD138, kappa, and lambda light chain markers were stained to diagnose and assert plasma cells and diversity of neoplastic PCM. It should be mentioned that immunohistochemical staining of CD138 was concentrated in the membrane, while kappa and lambda light chains were stained in the nucleus, cytoplasm, and membrane. In that line, 20% staining was considered as positivity for lambda and kappa markers, and regarding the severity of staining, tissue slides were divided into kappa and lambda types [Figure 1].

Patient samples chosen to analyses were 51 men (56.7%) and 39 women (43.3%). The mean and the range of age were about 65.6 years and 44–86, respectively, for PCM patients and were 42 years and 18–74, respectively, for reactive plasmacytosis. Of 45 PCM patients, 37 cases (82.2%) were κ type, and 8 cases (17.8%) were λ type [Figure 2a].

There were 26 men (70%) and 11 women (30%) in the kappa type group as well as 4 women (50%), and 4 men (50%) were lambda type [Figure 2b].

However, there was no significant relationship between the types of κ and λ with the sexuality of patients ($P > 0.05$). Of 37 patients in the kappa type group, 3 cases aged <50 years (8.1%), 21 cases with the age of 50–70 years (56.8%), and 13 cases were older than 70 years (35.1%). From 8 cases with lambda type, 3 cases aged between 50 and 70 years (37.5%) and 5 cases were over 70 years (62.5%), while no specimen was under 50 years old [Figure 2c].

After determining the ratio of κ/λ at the minimum error using R statistical software, the extent of κ/λ equal to × >3, × <0.48, and 0.48< × <3, respectively, was related to kappa type, lambda type and reactive plasmacytosis [Table 2a and b]. According to the mentioned equations in the section of materials and methods, the error rate of 0.011, the sensitivity of 100% and specificity of 97.8% were assessed to design the table.

Discussion

The morphological criteria used for the diagnosis of PCM are not always reliable since there is a great variation in the appearance of individual marrow particles.[14]

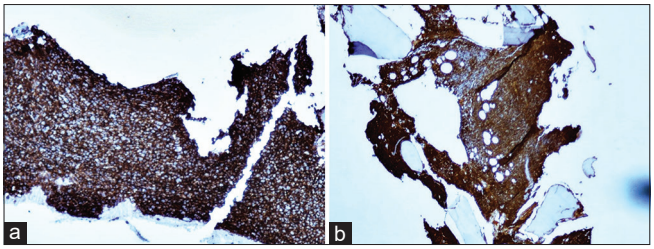

Figure 1: Immunohistochemical stains of bone marrow core biopsy showing diffuse positive staining for both kappa (a) and lambda (b)

PCM is considered as a clonal plasma cell disorder with neoplastic plasmacytosis in the bone marrow, which is different from reactive plasmacytosis.[15] There are valuable quantitative and qualitative morphological criteria to distinguish benign plasmacytosis from malignant plasmacytosis. In fact, it is impossible to differentiate PCM from other blood malignancies due to significant changes in the appearance of individual marrow particles.[14,16-18] Therefore, morphological evaluation cannot be used as a solitary way to differentiate PCM cells from reactive plasmacytosis or other hematocytes. In this line, IHC staining has been suggested to facilitate-specific diagnosis of plasma cells, especially in cases involved in bone marrow as well as alternative cases including non-secretory myeloma.[9,19,20]

A large body of evidence has been demonstrated that recognition markers such as CD38 and CD138 play an essential role to distinguish plasma cells from other hematocytes.[21] Some of the phenotypic characteristics of normal plasma cells, such as CD38 expression maintain by neoplastic cells.[22] Therefore, CD38-positive plasma cells

Table 1a: Frequency calculation pattern of x=K/L ratio in two groups of patients with myeloma and reactive plasmacytosis

	x < l	l ≤ x ≤ u	u < x	Total
Plasma cell myeloma	n_a	n_b	n_c	45
Reactive plasmacytosis	n_d	n_e	n_f	45

Table 1b: Frequency table 2×2 of x=K/L ratio in two groups of patients with myeloma and reactive plasmacytosis

	x < l or x > u	l ≤ x ≤ u	Total
Plasma cell myeloma	$n_a + n_c$	n_b	45
Reactive plasmacytosis	$n_d + n_f$	n_e	45

(1) Error rate = $\frac{n_b + n_d + n_f}{90}$, (2) Sensitivity = $\frac{n_a + n_c}{n_a + n_b + n_c}$,

(3) Specificity = $\frac{n_e}{n_e + n_d + n_f}$

Table 2a: Frequency of x=K/L ratio in the two groups of patients with plasma cell myeloma and reactive plasmacytosis

	x <0.48	0.48≤ x ≤3	3< x	Total
Plasma cell myeloma	8	0	37	45
Reactive plasmacytosis	1	44	0	45

Table 2b: Frequency 2×2 table of x=K/L ratio in two groups of patients with plasma cell myeloma and reactive plasmacytosis

	x <0.48 or x >3	0.48≤ x ≤3	Total
Plasma cell myeloma	45	0	45
Reactive plasmacytosis	1	44	45

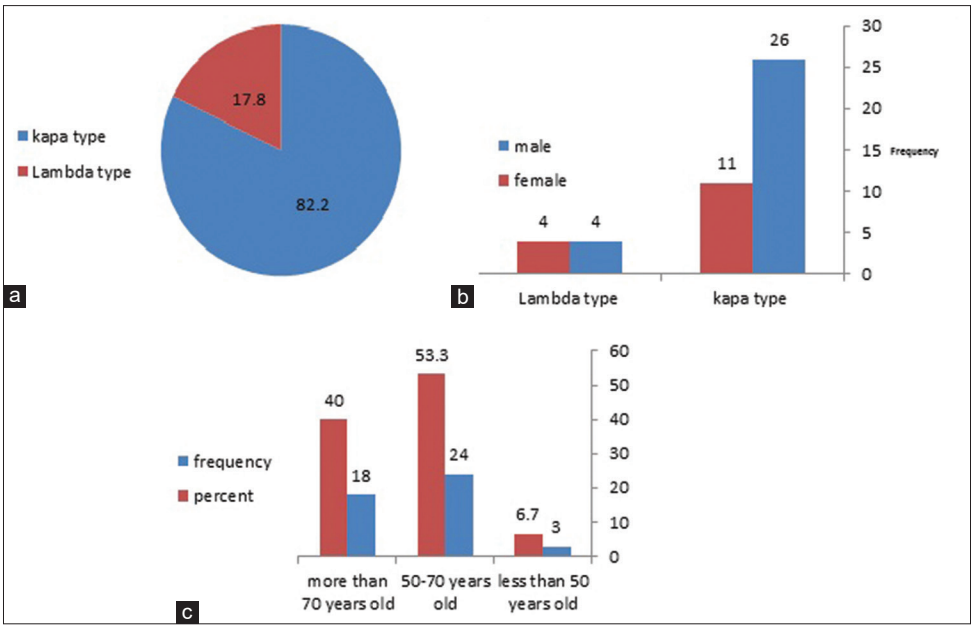

Figure 2: (a) Frequency of patients with plasma cell myeloma based on a subgroup (Kapa type and Lambda type). (b) Gender distribution of patients with plasma cell myeloma in subgroups (Kapa type and Lambda type). (c) Age distribution of patients with plasma cell myelomas

can be effective in diagnosing the malignant plasma cell disorder PCM.

It has been well known that the detection of immunoglobulin chains such as κ and λ light chains in serum, urine, and bone marrow are valuable methods in recognition of PCM cells.[22,23] In one study, the assessment of light chains of κ and λ in sera of patients and their ratio was associated with accurate recognition of PCM, especially nonsecretory type as well as affirmation of clonality.[24] Although the extent of light chains increases by aging, the ratio of κ/λ is independent with age, so the measurement of monoclonal κ/λ ratio is considered as an important diagnostic approach to detect monoclonal gammopathies including multiple myeloma.

The normal amount of light chain ratio is accepted as a range of about 0.26–1.65 in the sera of patients,[25] which is changed in general diseases such as immunosuppression and adrenal disorders.[26] Indeed, analysis of plasma cells, as well as κ and λ light chains in bone marrow using flow cytometry, IHC, immunofluorescence and *in situ* hybridization, is useful to assert clonality in plasma cells and PCM detection. In one study, flow cytometric evaluation of bone marrow biopsies in 27 PCM patients to detect monoclonality and neoplastic appearance (status), 26 patients showed monoclonality with the ratio between 0.76 and 1.5 which is differentiated from reactive plasmacytosis at a sensitivity and specificity of 96.3% 95.6%, respectively.[27] The analysis of κ and λ light chains and their ratio using IHC was investigated in two other studies: in one study, the ratio of $0.48<\kappa/\lambda<3$ with 94.7% sensitivity and 94.7% specificity as well as the ratio of $0.59<\kappa/\lambda<4$ with sensitivity of 100% and specificity of 97.8% in another study.[21]

In the present study, we also reviewed the ratio of κ and λ light chains for CD138 using IHC in 90 cases (45 PCM patients and 45 controls). All PCM patients showed monoclonality as well as the ratios of $\kappa/\lambda>3$ and $\kappa/\lambda<0.48$ in PCM patients with 100% sensitivity and 97.8% specificity which can be used to differentiate PCM patients from reactive plasmacytosis and is in line with other studies.

Conclusions

The analysis of κ and λ light chains using IHC could be used as a beneficial diagnostic way to detect PCM and differentiate it from plasmacytosis and other blood malignancies.

Financial support and sponsorship

This study was financially supported by Kermanshah University of Medical Sciences (Grant 93924).

Conflicts of interest

There are no conflicts of interest.

References

1. Boccadoro M, Pileri A. Plasma cell dyscrasias: Classification, clinical and laboratory characteristics, and differential diagnosis. Baillieres Clin Haematol 1995;8:705-19.
2. Kyle RA. Sequence of testing for monoclonal gammopathies. Arch Pathol Lab Med 1999;123:114-8.
3. Kyle RA, Child JA, Anderson K, Barlogie B, Bataille R, Bensinger W, *et al*. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: A report of the international myeloma working group. Br J Haematol 2003;121:749-57.
4. Rosai J. Rosai and Ackerman's surgical pathology e-book. 10 ed. china: Elsevier Health Sciences; 2011.

5. Jagannath S, Richardson P, Munshi NC. Multiple myeloma and other plasma cell dyscrasias. Cancer Management. 2011:1-20.
6. Anderson RE, Ishida K, Li Y, Ishimaru T, Nishiyama H. Geographic aspects of malignant lymphoma and multiple myeloma. Select comparisons involving Japan, England and the United States. Am J Pathol 1970;61:85-98.
7. Kumar V, Abbas AK, Aster JC. Robbins Basic Pathology. Canada: Elsevier Health Sciences; 2017.
8. Swerdllow S, Campo E, Harris NL. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues: France: IARC Press; 2008.
9. Pileri S, Poggi S, Baglioni P, Montanari M, Sabattini E, Galieni P, *et al.* Histology and immunohistology of bone marrow biopsy in multiple myeloma. Eur J Haematol Suppl 1989;51:52-9.
10. Al-Quran SZ, Yang L, Magill JM, Braylan RC, Douglas-Nikitin VK. Assessment of bone marrow plasma cell infiltrates in multiple myeloma: The added value of CD138 immunohistochemistry. Hum Pathol 2007;38:1779-87.
11. Bataille R, Jégo G, Robillard N, Barillé-Nion S, Harousseau JL, Moreau P, *et al.* The phenotype of normal, reactive and malignant plasma cells. Identification of "many and multiple myelomas" and of new targets for myeloma therapy. Haematologica 2006;91:1234-40.
12. Lima M, Teixeira Mdos A, Fonseca S, Gonçalves C, Guerra M, Queirós ML, *et al.* Immunophenotypic aberrations, DNA content, and cell cycle analysis of plasma cells in patients with myeloma and monoclonal gammopathies. Blood Cells Mol Dis 2000;26:634-45.
13. Reid S, Yang S, Brown R, Kabani K, Aklilu E, Ho PJ, *et al.* Characterisation and relevance of CD138-negative plasma cells in plasma cell myeloma. Int J Lab Hematol 2010;32:e190-6.
14. Canale DD Jr. Collins RD. Use of bone marrow particle sections in the diagnosis of multiple myeloma. Am J Clin Pathol 1974;61:382-92.
15. Bartl R, Frisch B, Burkhardt R, Fateh-Moghadam A, Mahl G, Gierster P, *et al.* Bone marrow histology in myeloma: Its importance in diagnosis, prognosis, classification and staging. Br J Haematol 1982;51:361-75.
16. Zervas K, Constantinou N, Karakantza M, Tsigalidou-Balla V. Anaplastic myeloma. Leuk Lymphoma 1995;16:515-9.
17. Sun NC, Fishkin BG, Nies KM, Glassy EF, Carpentier C. Lymphoplasmacytic myeloma: An immunological, immunohistochemical and electron microscopic study. Cancer 1979;43:2268-78.
18. Schneider DR, Taylor CR, Parker JW, Cramer AC, Meyer PR, Lukes RJ. Immunoblastic sarcoma of T – And B-cell types: Morphologic description and comparison. Hum Pathol 1985;16:885-900.
19. Taylor CR, Russell R, Chandor S. An immunohistologic study of multiple myeloma and related conditions, using an immunoperoxidase method. Am J Clin Pathol 1978;70:612-22.
20. Wei A, Juneja S. Bone marrow immunohistology of plasma cell neoplasms. J Clin Pathol 2003;56:406-11.
21. Nakayama S, Yokote T, Hirata Y, Iwaki K, Akioka T, Miyoshi T, *et al.* Immunohistological analysis in diagnosis of plasma cell myeloma based on cytoplasmic kappa/lambda ratio of CD38-positive plasma cells. Hematology 2012;17:317-20.
22. Kyle RA. "Benign" monoclonal gammopathy – After 20 to 35 years of follow-up. Mayo Clin Proc 1993;68:26-36.
23. Jackson N, Ling NR, Ball J, Bromidge E, Nathan PD, Franklin IM. An analysis of myeloma plasma cell phenotype using antibodies defined at the IIIrd international workshop on human leucocyte differentiation antigens. Clin Exp Immunol 1988;72:351-6.
24. Piehler AP, Gulbrandsen N, Kierulf P, Urdal P. Quantitation of serum free light chains in combination with protein electrophoresis and clinical information for diagnosing multiple myeloma in a general hospital population. Clin Chem 2008;54:1823-30.
25. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, *et al.* Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: Relative sensitivity for detection of monoclonal light chains. Clin Chem 2002;48:1437-44.
26. Pattenden RJ, Rogers SY, Wenham PR. Serum free light chains; the need to establish local reference intervals. Ann Clin Biochem 2007;44:512-5.
27. Nakayama S, Yokote T, Hirata Y, Iwaki K, Akioka T, Miyoshi T, *et al.* An approach for diagnosing plasma cell myeloma by three-color flow cytometry based on kappa/lambda ratios of CD38-gated CD138(+) cells. Diagn Pathol 2012;7:131.