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Original article

Pathological features of classical polyarteritis nodosa: Analysis of 19 autopsy cases

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ABSTRACT

Classical polyarteritis nodosa (cPN) is a rare autoimmune disease featuring systemic inflammation of middle- and small-sized arteries. Because most of autopsy cases underwent clinical treatment, arterial fibrinoid necrosis, which is the most specific finding of cPN, is often obscure. The aim of this study was to seek morphological characteristics of the middle-sized arteries in autopsy cases of cPN, and to identify immunohistochemical markers for the diagnosis of cPN. Nineteen patients who had undergone autopsy with a diagnosis of cPN were enrolled. Twenty-one autopsy cases without cPN were examined as control group. Arterial fibrinoid necrosis in medium-sized arteries was observed in 8/19 autopsy cases. Elastica van Gieson staining showed an increased number of elastic fiber layers ($P < 0.0001$) and greater distances between elastic fiber layers ($P < 0.0001$) in the renal middle-sized arteries of the cPN group. These findings probably reflected the post-inflammatory remodeling process after necrotizing vasculitis. On immunohistochemical examination, the cPN group showed high matrix metalloproteinase-1 and tumor necrosis factor-α expressions and decreased smoothelin expression in the vascular wall compared to the control group. When uncertain or atypical autopsy cases of cPN are examined, these findings can help to make the pathological diagnosis of cPN.

Introduction

Systemic vasculitis is a group of diverse conditions characterized by inflammation of blood vessels. Many classification schemes have been proposed, but the 1990 American College of Rheumatology criteria [8] and the 1994 Chapel Hill definitions for systemic vasculitis are the most widely used ones [9]. The Chapel Hill definitions distinguished classical polyarteritis nodosa (cPN) from the conditions previously classified as polyarteritis nodosa, such as microscopic polyangiitis (mPA), Wegener's granulomatosis [19] and Churg–Strauss syndrome [11], and defined cPN as systemic inflammation of the middle- and small-sized arteries without glomerulonephritis or vasculitis in arterioles, capillaries, or venules. However, the Chapel Hill definitions cannot be used as classification criteria in clinical practice. Therefore, several groups have tried to develop classification criteria, particularly for the small and medium vessel vasculitis, based on the Chapel Hill definitions [10,14,18].

The Japanese Intractable Vasculitis Study Group of the Ministry of Health, Labor and Welfare (MHLW) proposed a revised version of the criteria for clinical diagnosis of cPN in 2006 (Table 1) [20]. The definite diagnosis of cPN was made in patients with more than two major clinical findings and the histological findings. These MHLW criteria also indicate a probable diagnosis in a patient with more than two major clinical findings and angiographic findings, or in patients with more than six major clinical findings including fever and weight loss.

Renal biopsy specimens are often submitted for histological examination for the assessment of middle- and small-sized arteries in patients with clinically suspected cPN. However, the histological findings of cPN change over time, and they are classified into Stages I–IV (Stage I, degenerative stage; Stage II, acute inflammatory stage; Stage III, granulation tissue stage; and Stage IV, scar stage) according to the criteria proposed by Arkin [2]. Therefore, the main histological finding of cPN, necrotizing arteritis characterized by fibrinoid necrosis in the tunica media, does not always appear in these biopsy samples. Furthermore, inflammation of arteries is often masked in patients who have undergone therapeutic administration of glucocorticoid or cyclophosphamide under a probable diagnosis of cPN. Because most autopsy cases are seen after treatment, the specific morphological characteristics of middle- and small-sized arteries of cPN are often obscure, and no immunohistochemical marker for cPN has yet been identified.

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Table 1
Diagnostic features of classical polyarteritis nodosa (cPN).[a]

(1) Major clinical findings
(1) Fever and weight loss
(2) Hypertension
(3) Rapidly progressive renal failure, renal infarction
(4) Cerebral hemorrhage, cerebral infarction
(5) Myocardial infarction, ischemic heart disease, pericarditis, heart failure
(6) Pleurisy
(7) Gastrointestinal hemorrhage, intestinal infarction
(8) Mononeuritis multiplex
(9) Subcutaneous nodules, skin ulcers, gangrene, purpura
(10) Polyarthralgia, myalgia, muscular weakness
(2) Histological findings
Fibrinoid necrosis in medium- and small-sized arteries
(3) Angiographic findings
Multiple microaneurysms, stenoses, and occlusions in branches of the abdominal aorta (characteristically in renal arterioles)

[a] Based on criteria of the Japanese Intractable Vasculitis Study Group of the Ministry of Health, Labor and Welfare (MHLW).

The aim of this study was to determine the morphological characteristics of middle- and small-sized arteries of autopsy cases of cPN, and to identify any immunohistochemical marker for the diagnosis of cPN.

Materials and methods

Patients

A total of 19 patients who had undergone autopsy with a clinical diagnosis of cPN between 1984 and 2003 at Saga University Hospital were enrolled. A total of 21 autopsy cases without cPN or autoimmune diseases were also examined as a control group. The control group contained no hypertension case. Informed consent for the use of autopsy specimens and clinical information were obtained, and the study protocol was approved by the Ethics Committee of the Faculty of Medicine at Saga University. The consistency of clinical diagnosis of each case was re-evaluated according to WHLW criteria. Cases with mPA, Wegener's granulomatosis and Churg–Straus syndrome were excluded from this study.

Morphological assessment of the arteries

Morphological assessment of 4 μm-thick sections from formalin-fixed, paraffin-embedded kidney specimens was conducted after staining with hematoxylin and eosin (HE) and elastica van Gieson (EVG) stains. The glass slides of EVG staining were captured and converted into digital slides by a digital slide scanner (Nano Zoomer, Hamamatsu Photonics, Shizuoka, Japan). The thickness of the tunica intima and the number and each distance of elastic fiber layers at tunica intima of the middle-sized arteries in kidney were measured on digital slides by the software (NDP viewer, Hamamatsu Photonics, Shizuoka, Japan). Five middle-sized arteries of the kidney were selected on digital slides, and the mean number and distance of elastic fiber layers were evaluated in each case. The mean distance and the mean number of elastic fiber layers were compared between the cPN group ($n = 19$) and the control group ($n = 21$).

Immunohistochemistry (IHC)

Sections cut from formalin-fixed, paraffin-embedded tissue blocks were used. The dilutions of primary antibody and antigen retrieval methods are listed in Table 2. Normal mouse serum was used for negative control. The Envision system (Dako Cytomation, Glostrup, Denmark) was used as the second antibody. The slides were visualized by diaminobenzidine tetrahydrochloride, and the nuclei were counterstained with hematoxylin. The automatic stainer, Autostainer plus R (Dako Cytomation), was used for staining. Endothelial markers (CD31, CD34, CD105, and von-Willebrand factor (vWF)), myogenic markers (α-smooth muscle actin (α-SMA), smoothelin, h-caldesmon, myosin heavy chain, myogenin, and desmin), tumor necrosis factor (TNF)-α, matrix metalloproteinases (MMP-1 and MMP-8), and collagens (type I and type III), which are considered possible candidates as markers of cPN, were examined. The results of immunohistochemical analysis were evaluated in the whole arterial wall, including the adventitia. The intensity of immunoreactive products was assessed as none, weak, intermediate, and strong, and scored as 0, 1, 2 and 3, respectively. The IHC slides were assessed on light microscopy by two pathologists. The mean IHC scores were compared between the cPN group ($n = 19$) and the control group ($n = 21$).

Statistical analysis

Statistical analyses were performed using JMP version 8 software (SAS Institute, Cary, NC, USA). Statistical analysis to compare the two groups was performed using Student's *t*-test. A value of $P < 0.05$ was considered significant.

Table 2
Antibodies and conditions for immunohistochemistry.

Antibody	Clone	Source	Dilution	Retrieval
Collagen type I	COL-1	Abcam	1:100	MW
Collagen type III	CSI007-01	BioPorto	1:50	MW
Matrix metalloproteinase-1	Ab-6	Thermo	1	MW
Matrix metalloproteinase-8	RA-1-25210	Thermo	1:1000	PC
Tumor necrosis factor α	28401	R&D	1:50	MW
α-Smooth muscle actin	IA4	Dako	1:200	MW
Smoothelin	R4A	Abcam	1:100	None
h-caldesmon	h-CD	Dako	1:50	MW
Myosin Heavy Chain	SMMS-1	Dako	1:100	MW
Myogenin	F5D	Dako	1:50	MW
Desmin	D33	Dako	1:50	MW
CD31	JC70A	Dako	1:30	MW
CD34	0BEnd/10	Novocastra	1:50	MW
CD105	4G11	Novocastra	1:30	MW
von-Willebrand factor	F8186	Dako	1:200	Protease K

Abcam: Abcam, Cambridge, UK; BioPorto: BioPorto, Gentofte, Denmark; Thermo: Thermo Fisher Scientific, Waltham, MA, USA; R&D: R&D systems, Minneapolis, MN, USA; Dako: Dako Cytomation, Glostrup, Denmark; Novocastra: Novocastra, Leica Biosystems, Newcastle, UK; MW: microwave; PC: pressure cooker.