Supplementary methods

Serum protein electrophoresis

Protocol steps, reagents and suppliers. i) Protocol steps. a) Electrophoresis tank preparation. Barbital buffer solution (pH 8.6) was injected into both sides of the electrophoresis tank, the liquid level was adjusted to horizontal and a filter paper bridge was formed to connect the tank bodies on both sides. Cellulose acetate film (2x8 cm) was soaked in buffer solution for 30 min until completely wet, and then excess liquid was removed. b) Sampling: 2.5 μl serum was dipped into a micro sampler or cover glass, and dotted vertically at a distance of 1.5-2 cm from one end of the film's matte surface to form a uniform fine line. c) Electrophoresis: The dotted thin film sample was placed facing downwards on the filter paper bridge of the electrophoresis tank, and then the electricity was turned on after 5 min of equilibrium. The voltage was set at 120V, current at 0.5 mA/cm and the time at 50 min. d) Staining and rinsing: After electrophoresis, the film was immersed in amino black 10B staining solution (containing acetic acid and methanol) for 10 min, then rinsed with rinsing solution (ethanol, acetic acid and water) until the background was colorless. e) Quantitative analysis: Each protein band (albumin, α1, α2, β and γ globulin) and a blank comparison were cut off, 0.4 mol/l NaOH solution added for extraction and absorbance measured by 620 nm colorimetry, before calculating the percentage of each component.

ii) Reagents and suppliers. a) Barbital buffer solution (pH 8.6) was supplied by Sigma-Aldrich; Merck KGaA. Sodium barbiturate (12.76 g) and barbital (1.66 g) were dissolved in 1,000 ml distilled water. b) Amino black 10B staining solution was supplied from Beijing Baiaolaibo Technology Co., Ltd.; 0.5 g of amino black 10B, 10 ml of glacial acetic acid and 50 ml of methanol were dissolved in 40 ml of distilled water. c) Rinse solution was supplied by National Pharmaceutical Group Chemical Reagents; Sinopharm Chemical Reagent Co., Ltd.: 45 ml of 95% ethanol and 5 ml of glacial acetic acid were dissolved in 50 ml of distilled water. d) Eluent was supplied from National Pharmaceutical Group Chemical Reagents; Sinopharm Chemical Reagent Co., Ltd.: 0.4 mol/l NaOH solution.

Results. Table SI shows the test results of serum protein electrophoresis. The albumin and β1 globulin levels were slightly lower than the normal range, and the β2 globulin level was significantly higher than the normal range.

Immunofixation electrophoresis

Protocol steps, reagents and suppliers. i) Protocol steps. a) Sample preparation: The serum sample was diluted proportionally with diluent solution (if the total immunoglobulin level was high, the dilution was doubled). The sampling comb was used to load the sample into the agarose gel swim lane, with 10 μl sample added to each hole, and then the sample was placed in the wet box for 5 min. b) Electrophoretic separation: The gel sheet was set in the electrophoresis tank, then buffer (pH 8.6 barbital buffer) added and the electrophoresis procedure selected. The voltage was set at 0-300V, the current at 0-500 mA and the electrophoresis time at 50 min. c) Antibody incubation and fixation: After electrophoresis, the buffer strip was removed, the premixed antiserum (including IgG, IgA, IgM heavy chain and κ/λ light chain antibodies) was added to the swimming lane on the surface of the gel, and incubation was performed at 37˚C for 30 min, to make the antigen antibody complex form a sediment. d) Washing and staining: Washing solution (containing sodium azide) was used to remove unbound antibodies, followed by immersing in staining solution (Coomassie Brilliant Blue) for 15 min to color, and then decolorization until the background was clear. E) Result analysis: After drying the gel tablet, the band position was observed and compared with the reference swimming lane (ELP swimming lane) to determine whether there was a monoclonal immunoglobulin (such as IgG-κ).

ii) Reagents and suppliers. a) Electrophoresis-related reagents: Agarose gel was used to separate proteins and was used together with buffer solution. The buffer solution was pH 8.6 barbital buffer solution (Sigma-Aldrich; Merck KGaA). The fixative was a solution containing citric acid or formaldehyde, which was used to immobilize proteins. b) Antibody and antiserum: The antiserum kit contained anti IgG, IgA, IgM, κ and λ antibodies. Dilution solution was used to adjust sample concentration and consisted of Tris-HCl buffer (pH 8.3), 0.5% bovine serum albumin and 0.05% bromophenol blue. c) Dyeing and decolorization solution were supplied from Shanghai Xinyu Biotechnology Co., Ltd.; Coomassie Brilliant Blue R250 or Amino Black 10B were used for color development. Decolorization solution: Methanol glacial acetic acid water mixture (75:5:20). The agarose gel and antiserum kit were supplied by Thermo Fisher Scientific, Inc. The fixative and dilution solution were supplied by Shanghai Xinyu Biotechnology Co., Ltd.

Results. Table SII shows the test results of immunofixation electrophoresis. The IgM-λ type M protein was positive, which indicated the presence of immunoglobulin produced by abnormal proliferation of monoclonal plasma cells or B lymphocytes, with a heavy chain of IgM type and a light chain of λ type. This result strongly suggested hematological tumors (such as Waldenström's macroglobulinemia and multiple myeloma).

Genetic mutation detection

Protocol steps, reagents and suppliers. Gene mutation was detected by droplet digital polymerase chain reaction (ddPCR) technology. ddPCR is a nucleic acid detection technology based on single molecule amplification, which achieves absolute quantification and high-sensitivity mutation detection by dividing the reaction system into tens of thousands to millions of droplets. The specific steps are as follows: a) Sample processing and DNA extraction. Sample for testing: Bone marrow puncture material. Extraction method: DNeasy Kit reagent (Qiagen Inc.) was used to extract DNA from bone marrow fluid. b) Primer and probe design. Specific design: Design primers for mutation sites to avoid non-specific amplification. Blocker technology: Adding Blocker sequences to suppress wild-type template amplification and enhance mutation detection specificity. Probe labeling: 6-carboxyfluorescein-labeled mutant probes and Hexadecimal-labeled wild-type probes were used to achieve dual channel detection. c) Reaction system configuration 20 μl formula: DNA template, 1-10 ng;

primers/probes, 0.9-10 μmol/l ddPCR SuperMix (containing Taq enzyme and dNTPs; Kanglang Biotechnology), plus water to 20 μl. d) Droplet generation and amplification: The QX200 droplet generator was used to divide the reaction solution into droplets and transfer them to a PCR plate. Hot cycle program: Pre-denaturation at 95˚C for 10 min for 45 cycles (15 sec at 95˚C + 1 min at 63°C), terminated at 4˚C. e) Droplet reading and data analysis: Droplet analyzers (QX200) were used to detect fluorescence signals one by one and count the proportion of positive droplets. Judgment criteria: ≥2 positive droplets were judged as mutation positivity (sensitivity of 0.01%).

Results. Table SIII shows the test results of genetic mutation detection. MYD88L265P gene mutation was detected as positive, which did not rule out Waldenström's macroglobulinemia or lymphoplasmacytic lymphoma.

Bone marrow histology morphology protocol

Protocol steps, reagents and suppliers. a) Fixed agent: Modified methanol acetic acid mixture (Shanghai Yuanmu Biotechnology Co., Ltd.) was used at a concentration of methanol:acetic acid of 3:1, at a temperature of 25˚C and with a duration of 5 min. b) Staining agent selection: Wright Giemsa staining was used. Rui's dye solution preparation (Nanjing Senbeijia Biotechnology Co., Ltd.) was applied: 1 g of Rui's dye powder + 500 ml of phosphate buffer solution (pH 6.4-6.8) was ground and let stand for 7 days. For Jimsa dye solution preparation, 0.3 g Jimsa dye powder + 500 ml methanol were dissolved and use. c) Staining steps: Wright's staining solution was dropped onto the smear and let stand for 5 min, before adding an equal amount of buffer solution (pH 6.4-6.8), slowly mixing and staining for 15 min. Running water was used for rinsing, before air drying and examination under a microscope. d) Slicing preparation: The thickness of the smear was ~2 μm, and the slide was pushed by placing bone marrow droplets on one end of the glass slide and tilting it at an angle of 30-45˚ to form a smear with clear head/body/tail and uniform thickness. The densely distributed area of the tail cells wass used for classification and counting. The smear area was ~1.5x3 cm, and the thickness of the cell layer was transparent with visible handwriting seen through it (~2 μm). e) Microscopic observation: A high-resolution imaging system optical microscope (Nikon Eclipse E600) was used, including a low-power mirror (10X/0.25 NA) for observing the degree of proliferation and distribution of megakaryocytes throughout the entire film (scale, 200 μm). Oil mirror (100X/1.25 NA): Classification and counting of nucleated cells (scale bar, 20 μm). f) Image acquisition: Image analysis software NIS Elements BR (Nikon Corporation) was used for cell proportion quantification and morphological parameter analysis.

Results

Cytological examination of the bone marrow smear. Table SIV shows the test results of bone marrow histology morphology. a) The specimen was well collected, smeared and stained. The granules were (++), and the oil stain was (++). b) Active proliferation with G=40%, E=17.5% and G/E=2.29/1 was determined. c) The proportion of granulocyte series was normal, cells at all stages were visible and no obvious morphological abnormalities were found. d) The proportion of red cells was normal, and the majority were young red cells in the middle and late stages. The shape and size of the mature red cells were similar, with arrangement in a coin-shape. e) The proportion of lymphocytes increased, indicating mature lymphocytes. Approximately 10% of the cells were abnormal lymphocytes, characterized by small cell bodies, dense nuclear chromatin and cytoplasm ranging from minimal to moderate. Some cells exhibited a blue cytoplasm with a foamy appearance, resembling plasma cells, while others had irregular cytoplasmic margins with spiky protrusions. f) The whole film had a total of 94 megakaryocytes without obvious morphological abnormalities. Platelets were distributed individually and in small clusters, and were visible. There were less abnormal platelets. g) The proportion of plasma cells was ~4%, and the cells were mature plasma cells, with a few vacuoles seen in some.

Flow cytometry of bone marrow

Protocol steps, reagents and suppliers. i) Sample collection and processing. Specimen preparation: Bone marrow fluid (obtained by iliac or sternotomy). Preparation of cell suspension: The bone marrow fluid was diluted with phosphate-buffered saline (PBS) and 2% fetal bovine serum (FBS), and the cancellous bone was repeatedly blown with a syringe to release bone marrow cells into PBS.

ii) Red blood cell lysis. Reagent selection: Red blood cell lysis buffer (containing NH_4Cl, $KHCO_3$ and EDTA; Gibco; Thermo Fisher Scientific, Inc.) rapidly dissolves red blood cells while retaining white blood cell activity. Low osmolarity lysis buffer (1X RBC lysis buffer; Aladdin Scientific) gently lyses red blood cells to reduce cell damage. Operation process: Precooled lysis buffer (2-3 times the volume of cell suspension) was added and incubated on ice for 5-10 min. Centrifugation was performed at 4˚C (300 x g) for 5 min before discarding the supernatant and washing twice with PBS.

iii) Cell staining and antibody incubation. Staining buffer consisted of PBS+2% FBS+0.1% NaN (reduces non-specific binding; Gibco; Thermo Fisher Scientific, Inc.). Antibody combination: A five-color method was used for antibody labeling. Surface markers used the CD45/SSC gating method, combined with CD19 (B cells), CD38 (plasma cells), CD138 (plasma cells) and CD56 (abnormal expression). Fluorescence-labeled antibodies such as CD4-FITC, CD8-PC5.5 and CD3-APC were used. Incubation was performed at 4˚ for 20-30 min in the dark, following pretreatment with Fc receptor blockers (such as Human Fc Block; BioLegend, Inc.).

iv) Flow cytometry detection. The test instrument used was a Beckman Coulter Navios, and test conditions were set as a laser at 488 nm (FL1/FL2), 633 nm (FL4) and 405 nm (FL5). Parameters obtained were as follows: FSC-Lin, SSC-Log, FL1-Log, FL2-Log, FL4-Log, FL5-Log and FL6-Log. Voltage parameters were FSC 500V, SSC 450V and FL1-FL6 adjusted according to antibodies (such as FL1 600V). Data collection: A total of 100,000 cells were collected, the threshold was set to FSC/SSC, and debris and dead cells were excluded (7AAD+, viability Dye; BD Biosciences). Storage data format: FCS 3.0.

v) Data analysis. The CD45/SSC set gate method was used, which identifies hematopoietic cells and excluded non-hematopoietic cells such as mesenchymal stem cells.

Abnormal clone detection: κ/λ light chain ratio analysis (κ: λ>3:1 or <0.5:1 indicates monoclonal activity). Phenotypic analysis: Hematopoietic stem cells, CD34+/CD117+/HLA-DR-; and plasma cells, CD38++/CD138+/CD45low/-. Antibody combinations include (purchased from Beckman Coulter, BD Pharmingen, BioLegend and SouthernBiotech): CD4-FITC/CD56-PE/CD3-APC/CD8-PC5.5/CD45-PC7, CD5-FITC/CD10-PE/CD20-APC/CD19-PC5.5, CD38-FITC/CD123-PE,HLA-DR-FITC/CD33-PE/CD34-APC/CD117-PC5.5, CD11b-FITC/CD13-PE/CD16-APC/CD15-PC5.5, CD36-FITC/CD11C-PE/CD64-APC/CD14-PC5.5, CD71-FITC/CD235a-PE/CD41-APC/7AAD, CD7-PE/CD2-PC5.5 and κ-FITC/λ-PE.

Results. Table SV shows the test results of bone marrow histology morphology analysis. Lymphocytes accounted for 13.72% of the nucleated cells, with T lymphocytes making up 49.69%, a decrease in proportion. The ratio of CD4+/CD8+ was 0.37, also showing a decrease, and no significant phenotypic abnormalities were observed. CD19+ B cells formed 32.78% of the lymphocytes, characterized by the phenotype of CD19+CD20+Lambda++Kappa-CD5-CD10-CD3-CD4-CD8-CD2-CD7-. Granulocytes accounted for 68.96% of the total, with normal proportions, and no significant phenotypic or percentage changes at any stage. Monocytes accounted for 5.34%, with no significant phenotypic abnormalities. Reticulocytes accounted for 7.82%, with normal proportions and no significant phenotypic abnormalities. CD34+CD117+ myeloid cells accounted for 0.47%, with a low proportion and no significant phenotypic abnormalities.

In conclusion, the results of flow cytometry showed that ~4.94% of the nucleated cells in the specimens were abnormal mature B cells with an abnormal immune phenotype, and CD5-CD10-B cell lymphoma was not excluded.

Bone marrow biopsy

Protocol steps, reagents and suppliers. i) Preoperative preparation. Patient evaluation: Blood routine, coagulation function and other tests were completed to exclude contraindications. Position selection: The prone position or lateral position were recommended, as it was easy to puncture the posterior superior iliac spine (preferred site) or anterior superior iliac spine. The procedure and risks were explained to the patient, and written informed consent was obtained.

ii) Puncture and sampling. Disinfection and anesthesia: After disinfection of the puncture site, 2% lidocaine was injected locally to infiltrate the periosteum. Puncture procedure: A bone marrow biopsy needle was vertically inserted into the bone surface and rotated into the bone marrow cavity (depth of 1-1.5 cm). After removing the core needle, a plastic cannula needle was inserted to obtain a bone marrow tissue strip (length of 1 cm). Sample processing: The tissue strip was fixed in 10% neutral formalin or Bouin fixative, and sent to the Department of Pathology for embedding and sectioning.

iii) Postoperative treatment. Bleeding was halted by applying pressure to the puncture site for 10-15 min and covering with sterile gauze. The patient was able to leave the hospital after 30 min without bleeding. Compensation monitoring including being aware of hematoma, infection and other risks, and instructing the patient to avoid strenuous exercise.

iv) Hematoxylin and eosin staining. Tissue were fixed in 4% neutral formaldehyde (24-48 h). Paraffin embedding or frozen sectioning was performed. Tissues were sliced to a thickness of 4-5 μm, before mounting and baking (60˚C for 30 min). Xylene I/II was applied for 10 min each for dewaxing, before applying an ethanol gradient (100% I/II, 95% I/II, 85% and 70%, for 2 min each for hydration). Hematoxylin staining was performed using 0.5% hematoxylin staining solution (or modified Mayer hematoxylin) for 5-10 min, prior to rinsing with running water. Differentiation was performed using 1% hydrochloric acid alcohol (1:1 hydrochloric acid + 75% ethanol) for 3-5 sec (to remove non-specific coloring), with flowing water returning to blue (ammonia or lithium carbonate solution). Eosin staining was peformed using 0.5% eosin aqueous solution (or water-soluble eosin) for 3-5 min, prior to rinsing with running water. Ethanol gradient dehydration was performed (70, 85, 95% I/II and 100% I/II, for 2 min each) prior to xylene transparency (5 min each for I/II) and neutral gum sealing.

vi) Combined experimental steps of immunohistochemistry and Congo red staining. a) Sample preparation and dewaxing. The tissue was fixed with neutral formaldehyde and embedded in paraffin, with a slice thickness of 4-5 μm. For dewaxing hydration, xylene I/II were applied for 10 min each, followed by anhydrous ethanol I/II for 5 min each, 95% ethanol for 3 min and 80% ethanol for 3 min. Distilled water immersion was applied for 2 min. b) For Congo red staining, slices were immersed in Congo red staining solution (0.5 g Congo red powder dissolved in 80 ml distilled water + 20 ml ethanol) and incubated at 25˚C for 20 min. Slices were quickly immersed in 80% ethanol with 1% NaOH solution for 10 sec, and rinsed with running water for 5 min until the background was clear. Hematoxylin redyeing was performed with Harris hematoxylin staining solution for 2 min, then returned to blue with running water for 10 min. Gradient dehydration was performed using anhydrous ethanol I/II/III for 5 min each, followed by Xylene transparency twice for 5 min and neutral gum sealing. c) Immunohistochemical staining: Antigen repair was performed in a microwave or enzymatic digestion (such as trypsin) was used to treat slices and expose antigen epitopes. Non-specific binding sites were blocked with 5% BSA or normal serum at room temperature for 10 min. Specific primary antibodies (including anti-amyloid light chain antibodies) were added and incubated overnight at 4˚C. HRP-labeled secondary antibodies (including anti-rabbit HRP) were added and incubated at room temperature for 30 min. Diaminobenzidine coloration (brownish yellow precipitate) was applied, with the reaction terminated by running water. Hematoxylin counterstaining took 1 min, prior to rinsing with running water (mild counterstaining of cell nuclei, distinguishing background). Dehydration and sealing was performed as aforementioned for hematoxylin and eosin staining.

v) Main reagents. a) Fixation fluid: 10% neutral formaldehyde solution (Sinopharm Chemical Reagents Co., Ltd.) was used for routine fixation of bone marrow tissue to retain cell morphology. Bouin solution (Sigma-Aldrich; Merck KGaG) is suitable for rapid fixation, to reduce tissue contraction, and is often used in plastic embedding. b) Decalcification solution (Sinopharm Chemical Reagents Co., Ltd.): 15% hydrochloric acid solution for rapid decalcification (30-40 min), suitable

for bone marrow tissue containing trabeculae. 1% ammonia water to neutralize the residual acid after decalcification, to prevent tissue damage. c) Staining reagents: Hematoxylin and eosin staining solution (Thermo Fisher Scientific, Inc.): Conventional hematoxylin and eosin staining to observe cell morphology. Congo red staining solution (1%) combined with amyloid protein β-folding for coloration, supplied by Shanghai Yuanmu Biotechnology Co., Ltd. Immunohistochemistry reagents included citrate buffer/EDTA (Beijing Solarbio Science & Technology Co., Ltd.), 3% H_2O_2 (Beijing Solarbio Science & Technology Co., Ltd.), specific antibodies (Abcam), HRP-labeled secondary antibody (Thermo Fisher Scientic, Inc.), a DAB colorimetric kit (Sigma-Aldrich; Merck KGaA) and hematoxylin staining solution (Beijing Solarbio Science & Technology Co., Ltd.).

Results. The tissue sample consisted of one piece of gray-yellow bone marrow tissue with a size of 0.2x0.2x0.1 cm, fully wrapped and decalcified.

The bone marrow tissue obtained via posterior superior iliac spine puncture biopsy and submitted for examination showed a reduced proportion of red blood cells, with a decrease in the proliferation of hematopoietic cells between trabeculae. The proportions of granulocytes, erythrocytes and megakaryocytes in the red blood cell series were normal, with mature cell morphology and a few scattered plasma cells. Hematoxylin and eosin staining showed malignant proliferation of primitive/immature cells and significant reduction of adipocytes. Congo red staining (+) exhibited an orange-red color, indicating the presence of amyloid-like substances. Supplementary immunohistochemical results showed κ (focal+) and λ (focal+) results.

Amyloid protein mass spectrometry spectrum

Protocol steps, reagents and suppliers. i) Sample preparation and treatment. Superficial abdominal wall fat tissue was selected for the biopsy. The samples were fixed in 10% neutral formalin, then embedded and stained with Congo red. Amyloid deposition was confirmed by observing the apple green birefringence under polarized light.

ii) Micro-cutting and enzymatic hydrolysis laser micro-cutting (LMD). A laser capture microscope (Leica LMD7000) was used to accurately cut the positive area of Congo red to avoid contamination of the surrounding tissues. The cut tissue was incubated overnight with trypsin solution (Trypsin Gold, Mass Spectrometry grade) at 37˚C to generate peptides.

iii) Liquid chromatography-tandem mass spectrometry. The peptide segments were separated by high-performance liquid chromatography (Vanquish; Thermo Fisher Scientific, Inc.), and multi-level mass spectrometry was performed using a tandem mass spectrometer (Orbitrap Fusion Lumos) to obtain the fingerprint spectrum of the peptide segments. Amyloid precursor proteins (such as TTR and AL light chain) were identified by comparing amyloidosis-specific databases with software such as Mascot and MaxQuant.

iv) Type classification. According to the ranking of mass spectrometry signal intensity, the main type of amyloid protein was determined (such as the highest proportion of AL peptide in AL amyloidosis). Cross validation was performed by combining the results of immunofixation electrophoresis or free light chain detection.

v) Reagents and suppliers. a) Methanol Congo red staining (Shanghai Yuanmu Biotechnology Co., Ltd.): Contains 0.5 g Congo red, 80 ml methanol and 20 ml glycerol, and is used for specific binding of amyloid fibrils. Puchtler alkaline Congo red kit (Shanghai Yuanmu Biotechnology Co., Ltd.): Contains a high concentration of sodium chloride to improve the selectivity of staining. b) Fixation and decalcification reagent (Sinopharm Chemical Reagents Co., Ltd.): 10% neutral formalin for tissue fixation; and 15% hydrochloric acid solution for rapid decalcification (30-40 min), which is suitable for samples containing trabeculae of bone. c) Enzymatic and mass spectrometry reagent (Beijing Genomics Institute): Trypsin Gold was used to hydrolyze proteins to produce peptides. A C18 desalting column was used to remove salt and impurities, ro purify the peptide. The mass spectrometry mobile phase was an acetonitrile/formic acid solution (0.1% formic acid water + acetonitrile gradient elution).

Results. As shown in Fig. 4, fibrinogen α chain (Fibα), the Ig light chain constant (Igλ C2), the Ig heavy chains Igμ, Igγ1 and Igα C1, Ig light chain κ C, lysozyme and gelsolin were detected. Among the currently known typing proteins, Fibα has the highest spectrum number. However, Fiba <β + y suggests that Fibα originates from blood contamination (α218, β294, γ188), indicating it is a non-deposited protein (Kidney Int Rep. 2019 Apr 15; 4(7):977-986).

Excluding Fibα, among other typing proteins, Igλ has the highest spectrum number. In addition, there are many IgM (Igγ/α/μ) heavy chains. Compared with heavy chains, light chains (especially λ type) are more likely to form amyloid fibrils due to their genetic sequence and high secretion characteristics (13). Thus, light chains are more prone to deposit in the cardiac position, leading to cardiac amyloidosis. In the present study, excluding Fibα, among other typing proteins, Igλ C2 had the highest relative abundance in the spectrum, with κ and λ values significantly higher than those in the normal ranges (Table I), indicating that the main type was AL amyloidosis.

Biopsy of abdominal wall fat tissue

Protocol steps, reagents and suppliers. i) Preoperative preparation. a) Patient evaluation was performed to confirm that there was no coagulation dysfunction, local infection or other contraindications, and written informed consent was provided. b) The patient was placed in a supine position, the abdomen was exposed and the puncture point was selected at 7-10 cm beside the umbilicus. c) Complex iodine or iodophor was used to disinfect the skin with the puncture point as the center, and sterile towels were applied. Local injection of 2% lidocaine infiltrative anesthesia to the subcutaneous tissue was applied (needle insertion in multiple directions, at least 3 times).

ii) Fat aspiration. The 18G needle was replaced with a 20-ml syringe (Bidi Medical), inserting the needle horizontally under negative pressure and adjusting the position of the needle tip to retract yellow adipose tissue. Multiple directions of repeated aspiration (5-10 times) were used to ensure that a sufficient sample was obtained (2-5 ml).

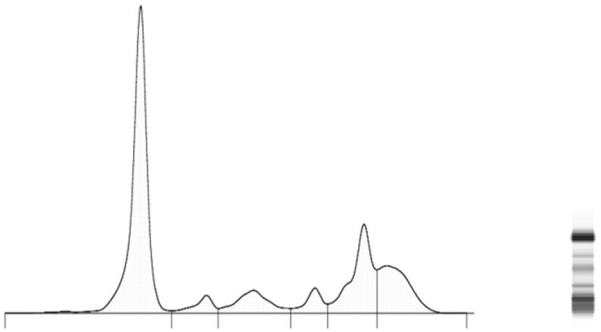
iii) Sample processing. The adipose tissue was transferred to 10% neutral formalin fixed solution (Sinopharm Chemical Reagents Co., Ltd.), and sent to the Department of Pathology for hematoxylin and eosin staining, Congo red staining and other tests.

iv) Postoperative treatment. After the removal of the needle, pressure was applied to the puncture point for 5-10 min, before covering with sterile dressing and observing the patient for 30 min without signs of bleeding.

v) Congo red staining. a) Slices were immersed in Congo red staining solution (0.5 g Congo red powder dissolved in 80 ml distilled water + 20 ml ethanol) and incubated at 25˚C for 20 min. b) Slides were quickly immersed in 80% ethanol with 1% NaOH solution for 10 sec, before rinsing with running water for 5 min until the background was clear. c) Hematoxylin redyeing was performed with Harris hematoxylin staining solution for 2 min, then the slides were returned to blue with running water for 10 min. Gradient dehydration was peformed using anhydrous ethanol I/II/III for 5 min each, followed by xylene transparency twice for 5 min and neutral gum sealing. Congo red staining solution (1%) was combined with amyloid protein β-folding for coloration (Shanghai Yuanmu Biotechnology Co., Ltd.). Fixed fluid and dehydrator consumables were supplied by Leica Microsystems.

Results. The specimen was fixed in formalin, and had the appearance of a pile of gray-red tissue with a volume of 0.8x0.6x0.2 cm. Using an ordinary light microscope, starch deposits appeared brick red, while collagen fibers were light pink. Using a polarized light microscope, positive areas exhibited apple green birefringence (diagnostic gold standard). The abdominal wall fat tissue had a small amount of adipose tissue and visible amyloid-like substances. Congo red-positive staining and apple green birefringence were exhibited, which confirmed cardiac amyloidosis in this case.

Figure S1. Test result of serum protein electrophoresis. The peak on the left represents ALB protein and the peak on the right represents γ globulin. The appearance of steep 'peaks' in the right elecrophoretic bands indicates the presence of abnormally increased immunoglobulin. Ref. Conc., reference concentration′ ALB, albumin.



Serum protein electrophoresis

Fractions	%		Ref. %	Ref. Conc.
ALB	49.2	<	>= 558.0	402.0–476.0
Alpha 1	3.5	<	29.0–49.0	21.0–35.0
Alpha 2	8.0	<	>=71.0	51.0–85.0
Beta 1	4.5	<	47.0–72.0	34.0–52.0
Beta 2	19.2	<	32.0–65.0	23.0–47.0
Gamma	15.6	<	>=111.0	8.0–135.0

A/G ratio: T.P.: g/dl

Figure S2. Low voltage in the limb leads in the electrocardiogram.

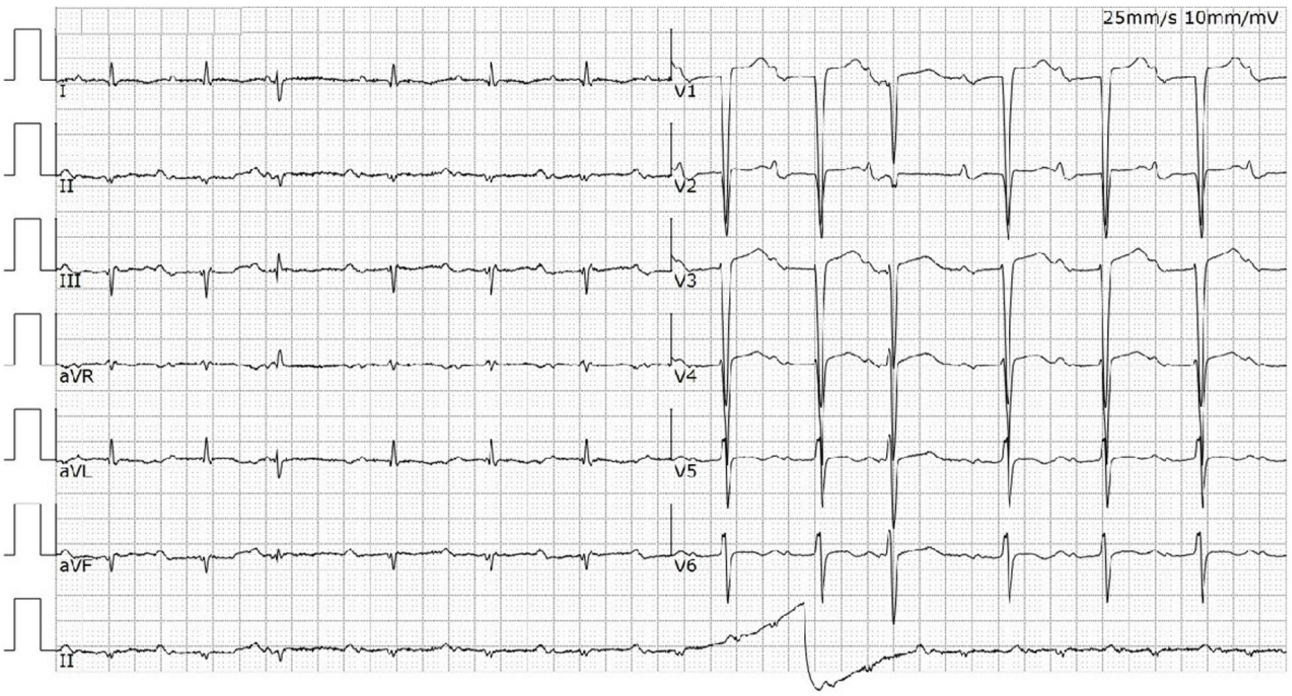


Figure S3. Gene mutation detection by droplet digital polymerase chain reaction. The blue dots represent the MYD88 mutant type. The green dots represent MYD88 wild-type (no mutation). Orange dots represent a mixed state of mutant and wild-type (such as chimeric mutations or low abundance mutations). The black dots represent the background noise.

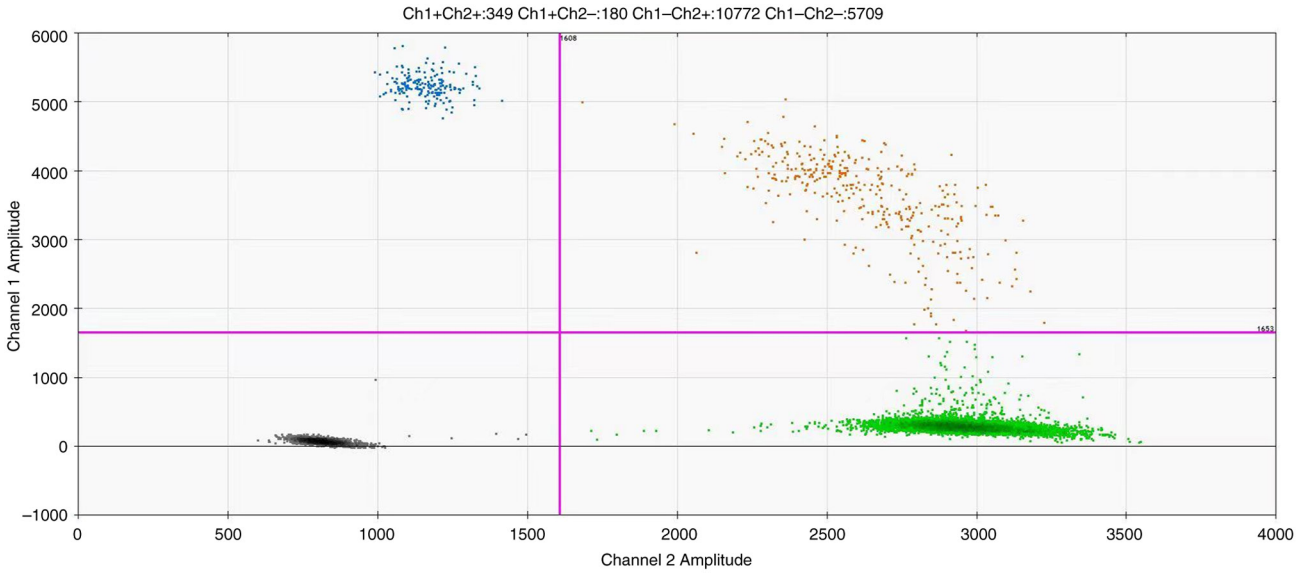


Figure S4. Flow cytometry results. The antibody labeling method is the five color method. The antibodies include CD19-PC5.5 for recognizing B cells, and CD20-APC for recognizing mature B cells. The gated area includes CD19+ and CD20+ cells (pink area), representing a subset of B cells that simultaneously express CD19 and CD20. The green area on the left represents CD19 low expression or negative cells. The 4.94% is the proportion of CD19+ and CD20+ in the total lymphocytes based on upstream lymphocyte gating. The 32.78% represents the proportion of CD19+ and CD20+ in CD19+ cells in this case.