Research Article

Bisalbuminemia: A rare finding identified via serum protein agarose gel electrophoresis and capillary electrophoresis techniques

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Keywords

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Abstract

Background: Bisalbuminemia is a rare abnormality characterized by detection of two distinct albumin bands or double peaks through serum protein agarose gel electrophoresis or capillary electrophoresis, suggesting the coexistence of normal and modified albumin in the same patient. However, it is challenging to visualize the double bands due to the limited resolution of agarose gel electrophoresis. Besides, capillary electrophoresis cannot directly visualize serum proteins.

Methods: Serum samples were obtained from the Hematology Hospital of the Chinese Academy of Medical Sciences and analyzed using agarose gel electrophoresis and capillary electrophoresis. Agarose gel electrophoresis and capillary electrophoresis were performed using a SPIFE4000 electrophoresis apparatus from Helena or Capillarys3 Tera system.

Results: Three samples of bisalbumin were detected in the albumin region in patients diagnosed with multiple myeloma via agarose gel electrophoresis and capillary electrophoresis techniques.

Conclusions: These findings suggest that capillary electrophoresis technology combined with traditional agarose gel electrophoresis methods can significantly improve the detection rate of bisalbuminemia. Serum dilution or depolymerization may be utilized to enhance detection when bisalbumin bands are not readily identifiable in agarose gel electrophoresis, thereby ensuring the accuracy of bisalbuminemia diagnosis.

Introduction

Human serum albumin is synthesized by hepatocytes in the liver. Serum albumin consists of a single polypeptide chain comprising 585 amino acids and has a molecular weight of 66.5 kDa. It has multiple physiological functions, including sustaining plasma colloid osmotic pressure, inhibiting the formation of oxidants, catalyzing biochemical reactions, and transporting endogenous and exogenous substances (thyroid hormones, steroids, and fatty acids) [1-2].

Clinical chemistry analyzers are widely used to quantify serum albumin in clinical practice. Besides, protein components of albumin are identified through serum protein electrophoresis. Agarose gel electrophoresis is one of the simplest and widely utilized methods for the separation of serum proteins in clinics due to its distinct banding patterns and high reproducibility. Using this technique, researchers can differentiate serum proteins on the basis of variations in their isoelectric points and molecular weights. The proteins can be grouped into several categories, including albumin, alpha-1 globulins, alpha-2 globulins, beta globulins, and gamma globulins. However, capillary electrophoresis provides rapid detection and enhanced resolution compared with traditional agarose gel electrophoresis due to the advancement of electrophoresis technology [3]. Capillary electrophoresis separates proteins based on their charges and sizes, resulting in varying migration velocities within an electric field [4]. The detector captures the migration speeds of various protein components, producing electropherograms with different electrophoretic peaks. The protein components, including albumin, alpha-1 globulins, alpha-2 globulins, beta-1 globulins, beta-2 globulins, and gamma globulins, can be identified by analyzing these peaks [5]. The detector can be used for serum protein electrophoresis and immunotyping detection in myeloma. Notably, the methods are critical for the screening and diagnosis of multiple myeloma.

Normal human serum albumin appears as a single band or a prominent electrophoretic peak during serum protein detection. However, two distinct bands may be detected in the albumin region in rare cases during serum protein agarose gel electrophoresis, a phenomenon known as bisalbuminemia [6]. This condition was first described in diabetic patients by Scheurlen in 1955, who exhibited two distinct bands with different electrophoretic mobilities on agarose gel electrophoresis [1]. Bisalbuminemia occurs in about 1 in 1000-1 in 3000 people. However, the incidence of bisalbuminemia among North American Indians is about 1 in 100 individuals [9]. Bisalbuminemia can either be hereditary or acquired [7,8]. Hereditary bisalbuminemia is a permanent condition inherited in an autosomal codominant manner. In contrast, acquired bisalbuminemia is associated with certain diseases, such as diabetes or multiple myeloma, or in patients treated with penicillin and other drugs[10]. In this study, double electrophoretic bands and peaks were detected in the albumin region of three patients diagnosed with multiple myeloma using serum protein agarose gel electrophoresis and capillary electrophoresis techniques. Compared with individual detection methods, the integration of the above techniques facilitates the rapid visualization of double protein bands and enhances the sensitivity of detecting bisalbumin and electrophoretic peaks. This comprehensive approach promotes accurate and timely identification of bisalbuminemia.

Materials and Methods

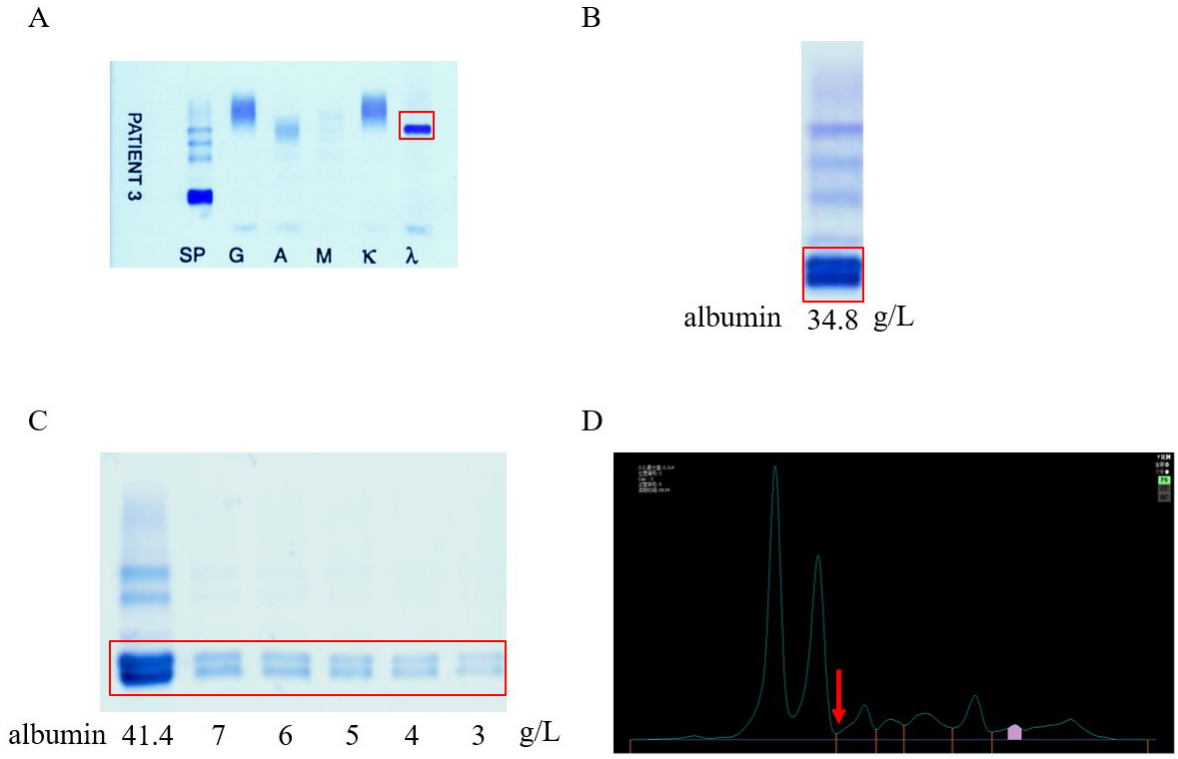
Three serum samples were obtained from the Hematology Hospital of the Chinese Academy of Medical Sciences and analyzed using agarose gel electrophoresis and capillary electrophoresis. Acquired causes of bisalbuminemia are not ruled out in the above cases. Agarose gel electrophoresis was conducted on the SPIFE4000 electrophoresis apparatus purchased from Helena to examine and results were quantified using an optical densitometer. Capillary electrophoresis was carried out using a fully automated Capillarys3 Tera system. The results were analyzed using PHORESIS software. Notably, serum samples were either diluted with normal saline or treated with ß-mercaptoethanol.

Results

Agarose gel electrophoresis and capillary electrophoresis can identify bisalbuminemia

An 82-year-old male patient with anemia admitted to our hospital was diagnosed with multiple myeloma following a bone marrow examination. Immunofixation electrophoresis identified a monoclonal lambda light chain component in the ß region (Figure 1A). Agarose gel electrophoresis detected double bands in the albumin region before treatment (Figure 1B). The patient underwent four cycles of the VRD (bortezomib, lenalidomide, and dexamethasone) regimen, resulting in a complete response. Subsequently, the patient received regular infusions of bisphosphonates to mitigate bone destruction and continued treatment with lenalidomide and ixazomib. However, treatment was adjusted to daratumumab upon re-evaluation due to disease progression. Agarose gel protein electrophoresis detected double bands in the albumin region during the treatment process. Notably, the bisalbumin bands were more distinct at a protein concentration of 7 g/L following concentration gradient dilution of the serum sample (Figure 1C). Similarly, capillary electrophoresis detected bisalbumin peaks (Figure 1D). These findings suggest that agarose gel electrophoresis and capillary electrophoresis can identify bisalbuminemia.

Figure 1: Agarose gel electrophoresis and capillary electrophoresis techniques detecting bisalbumin.

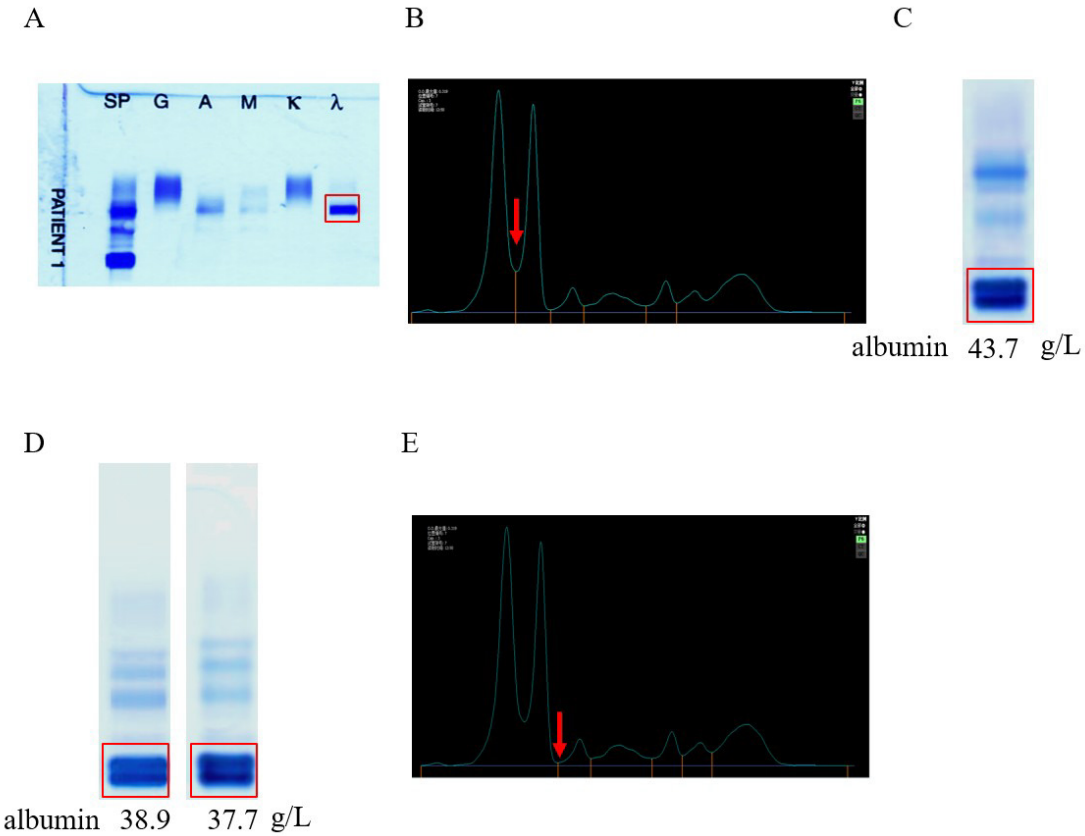


A. IFE showing a monoclonal light chain λ component in the ß region. B. Agarose gel electrophoresis showing double bands in the albumin fraction before treatment. C. Agarose gel electrophoresis showing double bands in the albumin fraction after treatment. D. Capillary electrophoresis showing double peaks after treatment.

Capillary electrophoresis alone can easily result in misjudgment, which can be corrected via agarose gel electrophoresis

A 44-year-old male patient with elevated serum uric acid and creatinine levels presented to the nephrology department due to an unusual oral odor. Serum immunofixation electrophoresis revealed the presence of precipitated bands in the lambda lane. Further evaluation through a whole-body CT scan confirmed the presence of myeloma lesions. Besides, immunofixation electrophoresis demonstrated a monoclonal light chain λ component in the γ region, thereby confirming the diagnosis of multiple myeloma (Figure 2A). The patient was treated with Daratumumab combined with pomalidomide, bortezomib, and dexamethasone (VPD) regimen. The patients received continuous treatment with regular follow-ups. Notably, the firstelectrophoretic peak and second peak were initially misidentified as albumin and alpha-1 globulins, respectively, during the diagnostic and treatment process via capillary electrophoresis (Figure 2B). However, we retrospectively reviewed the agarose gel electrophoresis data from pre- and post-treatment and identified distinct double bands in the albumin region (Figure 2C-2D). The results of capillary electrophoresis were corrected, revealing the presence of bisalbumin electrophoretic peaks (Figure 2E). Capillary electrophoresis can only display peak patterns. However, expertise is required to recognize the difference, making it challenging to identify bisalbumin peaks. In contrast, agarose gel electrophoresis can accurately display two bands in the albumin region, thereby facilitating a more precise bisalbuminemia diagnosis.

Figure 2: Capillary electrophoresis may lead to erroneous interpretations.

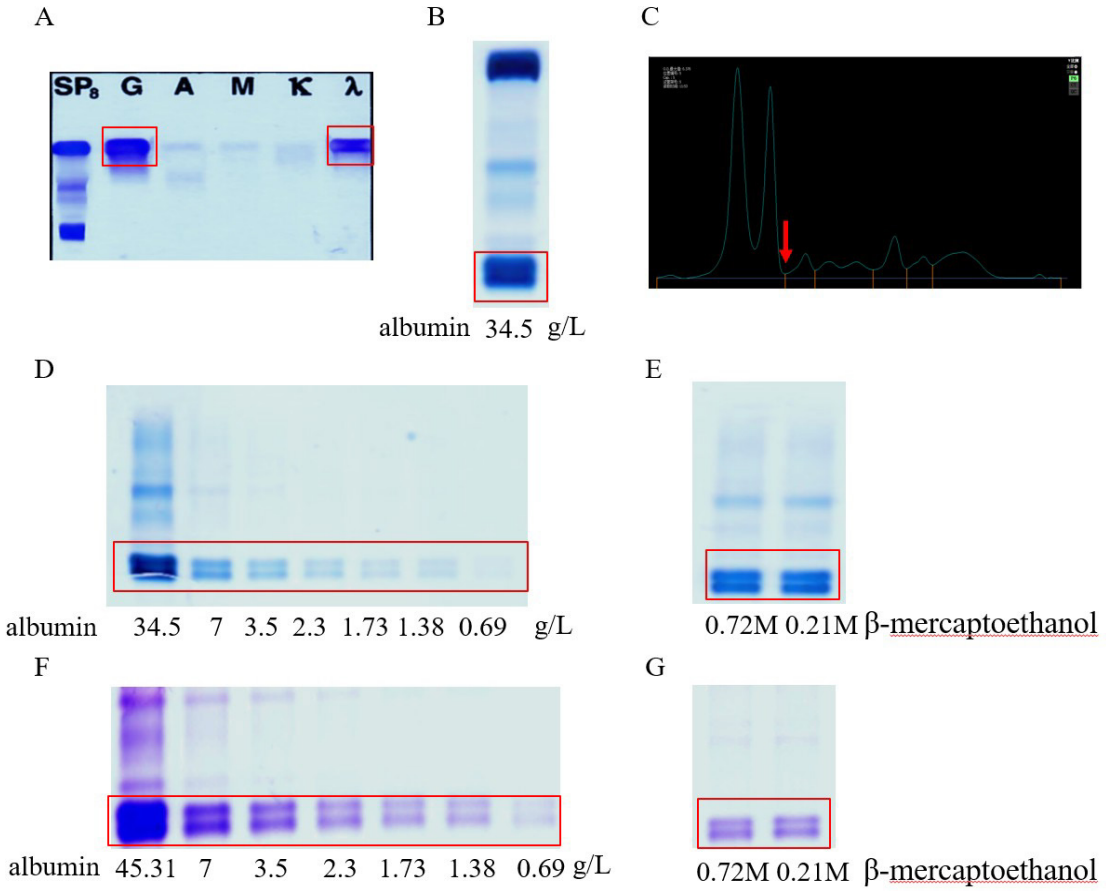


A. IFE showing a monoclonal light chain λ component in the γ region. B. The erroneous result of capillary electrophoresis where the initial electrophoretic peak was characterized as albumin, whereas the subsequent peak was classified as alpha-1 globulins after treatment. C. Agarose gel electrophoresis showing double bands within the albumin fraction before treatment. D. Agarose gel electrophoresis showing double bands within the albumin fraction after treatment. E. Capillary electrophoresis demonstrating the presence of double peaks in the albumin region after treatment.

The integration of agarose gel electrophoresis with capillary electrophoresis may facilitate a precise and expedited diagnosis of bisalbuminemia

A 49-year-old male patient with anemia, presented with foamy urine at a local hospital, and was diagnosed with multiple myeloma. The patient was admitted to our hospital for further evaluation and treatment. A thorough diagnostic evaluation, which included serum immunofixation electrophoresis, revealed the presence of a monoclonal IgG lambda protein in the γ region (Figure 3A). Key findings included an M-spike of 144.44%, an M-protein concentration of 48.806 g/L, and significantly elevated free lambda light chains at 4269.18 mg/L. These results indicate the presence of a monoclonal gammopathy, consistent with an abnormal proliferation of plasma cells. Further diagnosis was achieved through bone marrow aspiration and biopsy, flow cytometry, and screening for gene mutations associated with multiple myeloma. The patient underwent four cycles of chemotherapy utilizing a regimen with VPD. Agarose gel electrophoresis results revealed a concentrated band in the albumin region before treatment (Figure 3B). Additionally, capillary electrophoresis revealed the presence of double peaks in the albumin region (Figure 3C). The methodology was refined based on these observations. Quantitative analysis showed that the concentration of serum albumin was 34.5 g/L, with two distinct bands detected in the albumin region following concentration gradient dilution. The albumin bands were optimally separated at a serum albumin concentration of 7 g/L. However, the visibility of the bisalbumin bands diminished as the dilution ratio increased before treatment (Figure 3D). Protein depolymerization using 0.72 M and 0.21 M ß-mercaptoethanol corroborated this phenomenon. Notably, the depolymerization effect of ß-mercaptoethanol was not significantly different between the two concentrations (Figure 3E). We further employed agarose gel electrophoresis to analyze the serum samples obtained from patient after treatment and observed the same bisalbumin bands (Figure 3F-3G). These findings suggest that bisalbumin bands can be effectively observed through agarose gel electrophoresis at 7 g/L albumin concentration or when depolymerization is conducted using ß-mercaptoethanol at 0.72 M or 0.21 M. Therefore, the integration of agarose gel electrophoresis and capillary electrophoresis can provide a rapid and accurate diagnostic approach for bisalbuminemia.

Figure 3: The integration of agarose gel electrophoresis and capillary electrophoresis facilitates precise bisalbumin diagnosis.



A. IFE showing a monoclonal IgG lambda protein in the γ region.

B. Agarose gel electrophoresis demonstrating a concentrated band within the albumin fraction before treatment.

C. Capillary electrophoresis showing double peaks after treatment.

D. The quantified concentrations of albumin (34.5 g/L, 7 g/L, 3.5 g/L, 2.3 g/L, 1.73 g/L, 1.38 g/L and 0.69 g/L) before treatment.

E. Serum protein agarose gel electrophoresis performed after depolymerization using 0.72M and 0.21M β-mercaptoethanol before treatment.

F. The quantified concentrations of albumin (45.31 g/L, 7 g/L, 3.5 g/L, 2.3 g/L, 1.73 g/L, 1.38 g/L and 0.69 g/L) after treatment.

G. Serum protein agarose gel electrophoresis performed after depolymerization using 0.72M and 0.21M β-mercaptoethanol after treatment.

Discussion

Bisalbuminemia is characterized by the presence of two distinct albumin detected through serum protein electrophoresis, suggesting a variant albumin in the serum. This condition is associated with the occurrence of diverse diseases [11-14]. Compared with normal albumin, these variants exhibit functional alterations, such as decreased binding affinity to molecules (testosterone and bilirubin), a shorter half-life, and suppressed binding to warfarin. Notably, other variants demonstrate increased binding affinity to progesterone, triiodothyronine (T3), thyroxine (T4), and fatty acids [2,15]. However, acquiredbisalbuminemia occurrence is associated with various conditions, including diabetes, multiple myeloma, nephrotic syndrome, and chronic kidney disease [7,11,14,16].

Besides, the precise etiology of bisalbuminemia and its correlation with disease progression is unknown. Nonetheless, bisalbuminemia can complicate diagnoses derived from serum protein electrophoresis, which may result in diagnostic difficulties. Therefore, the identification of such variants during serum protein electrophoresis is important. Faporta et al. reported bisalbuminemia in a patient with multiple myeloma through routine gel electrophoresis analysis. Similarly, Hyung-Seok Yang et al. identified bisalbuminemia in patients with Parkinson's disease utilizing capillary electrophoresis, which could not be detected via agarose gel electrophoresis [17]. Capillary electrophoresis can reveal double electrophoretic peaks due to its superior sensitivity in differentiating variant albumin. Notably, agarose gel electrophoresis cannot detect double electrophoretic peaks.

Agarose gel electrophoresis is an easy-to-operate technique which can be utilized in routine laboratory activities. This method allows the direct examination of serum proteins through staining, improving disease detection. However, the resolution of agarose gel electrophoresis is inferior to that of capillary electrophoresis, limiting the separation of proteins with similar molecular weights. While gel images can be scanned for quantitative analysis, this approach has poor accuracy.

In contrast, capillaryelectrophoresis exhibit more optimized separation potential for proteins with comparable molecular weights, providing high resolution. The equipment contains

automated sample injection and data processing systems, which reduce human error. Nevertheless, operating and maintaining this equipment requires specialized tools and skilled personnel, which adds to the complexity. Moreover, the costs associated with the equipment and consumables can be significant, leading in higher overall expenses [18].

The low resolution of agarose gel electrophoresis limits the observation of the double bands. In contrast, capillary electrophoresis offers enhanced resolution, allowing for the detection of double peaks. However, the second electrophoretic peak is sometimes misidentified as alpha-1 globulins, which may lead to inaccurate diagnostic reports. Therefore, one method alone cannot accurately diagnose bisalbuminemia.

In this study, the integration of agarose gel electrophoresis with capillary electrophoresis facilitated the observation of distinct bands and separation peaks corresponding to the two albumin components. This approach significantly enhanced the detection rate of bisalbuminemia and improved the accuracy of the diagnostic report. Serum dilution or depolymerization is necessary for effective differentiation of the bisalbumin band when concentration bands appear in the albumin region during agarose gel protein electrophoresis. The molecular structure and characteristics of albumin molecules may be easily elucidated in the future as the detection rate of bisalbuminemia increases.

Conclusion

Bisalbuminemia is a rare non-pathological phenomenon that can be accurately detected through the integration of agarose gel electrophoresis and capillary electrophoresis. Notably, a concentration gradient dilution should be utilized to identify the appropriate serum concentration when the results of agarose gel electrophoresis are not easily distinguishable, allowing clearer visualization of the albumin bands. The integration of these two techniques offers a more accurate screening method for bisalbuminemia, thereby enhancing the precision of disease monitoring.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

All authors contributed to the study conception and design. Conceptualization, Data curation, Formal analysis, Writing-original draft were performed by Qian Ren. Conceptualization, Data curation, Writing-original draft were performed by Tong Liu. Conceptualization, Writing-original draftwere performed by Jing Li. Methodology, Writing-review & editing were performed by Yulong Fan, Shoulei Wang, Lele Wang, Yansheng Wang and Zhaojing Liu. Supervision, Writing-review & editing were performed by Yonghui Xia. Supervision, Writing-review & editing were performed by Guoqing Zhu. Project administration, Supervision, Writing-review & editing were performed by Yansong Ren. All authors read and approved the final manuscript.

Data statement

Data will be made available on request.

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Data availability

Data will be made available on request.

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