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# "Composite" lymphoma, lymphoplasmacytoid and diffuse large B-cell lymphoma of the spleen: molecular-genetic evidence of a common clonal origin

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**Abstract** We describe here the first well-characterized case of "composite" lymphoma of the spleen in which the two components were a low-grade and a high-grade B-cell non-Hodgkin's lymphomas. The patient was an elderly man with prominent splenomegaly and multiple hypoechogenic lesions of the spleen. A splenectomy was performed, and the macroscopic and histological findings showed the simultaneous presence of a "low-grade" B-cell lymphoma, lymphoplasmacytoid (immunocytoma) and a "high-grade" B-cell lymphoma (immunoblastic), which were spatially separated. The two lesions expressed the same immunoglobulin light chain (lambda), but the Southern blot analysis showed different patterns of immunoglobulin heavy chain (IgH) clonal rearrangement. PCR analysis followed by direct sequencing of the IgH-amplified rearrangement products provided molecular-genetic evidence that the two components of the composite lymphoma had the same clonal origin. Since both EBV LMP-1 and p53 were negative by immunohistochemistry, it is unlikely that EBV and p53 were involved in the neoplastic progression in this case. PCR analysis and direct sequencing of IgH-amplified rearrangement products are useful tools to investigate clonality in cases in which Southern blot analysis cannot be performed or does not provide conclusive findings.

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## Introduction

The simultaneous occurrence of two distinct types of lymphoma in the same site is defined as "composite" lymphoma [15, 22]. In some cases one component of the lesion is Hodgkin's disease and the other non-Hodgkin's lymphoma [10, 11]. In others the two components are both non-Hodgkin's lymphomas with different morphological features [15, 22]. Composite lymphomas most commonly occur in lymph nodes, but cases of such tumours arising in extranodal sites have also been described [1, 16]. In every case of composite lymphoma a major point of interest is whether the two components are clonally related or not; this problem usually requires a multidisciplinary approach based on different techniques.

In this paper we report on a well-characterized case of composite lymphoma of the spleen featured by the coexistence of a low-grade and a high-grade non-Hodgkin's lymphoma, which has not previously been described. Immunohistochemistry, Southern blot hybridization, and PCR analysis of immunoglobulin heavy chain (IgH) followed by direct sequencing of the amplified products were used to investigate the clonality of the two different components of this lesion.

## Case report

A 77-year-old man was admitted to our Institution on April 1997 because of abdominal pain and severe splenomegaly. The haemogram was within normal limits, but LDH was high (1422). Abdominal ultrasound showed an enlarged spleen (242 mm) featured by the presence of multiple hypoechogenic roundish lesions from 41 mm to 124 mm in diameter. A CT scan confirmed this finding. A bone marrow smear was negative, but a bone marrow core biopsy was consistent with a diagnosis of B-cell lymphoproliferative disorder, NOS. On these grounds, a splenectomy was performed in

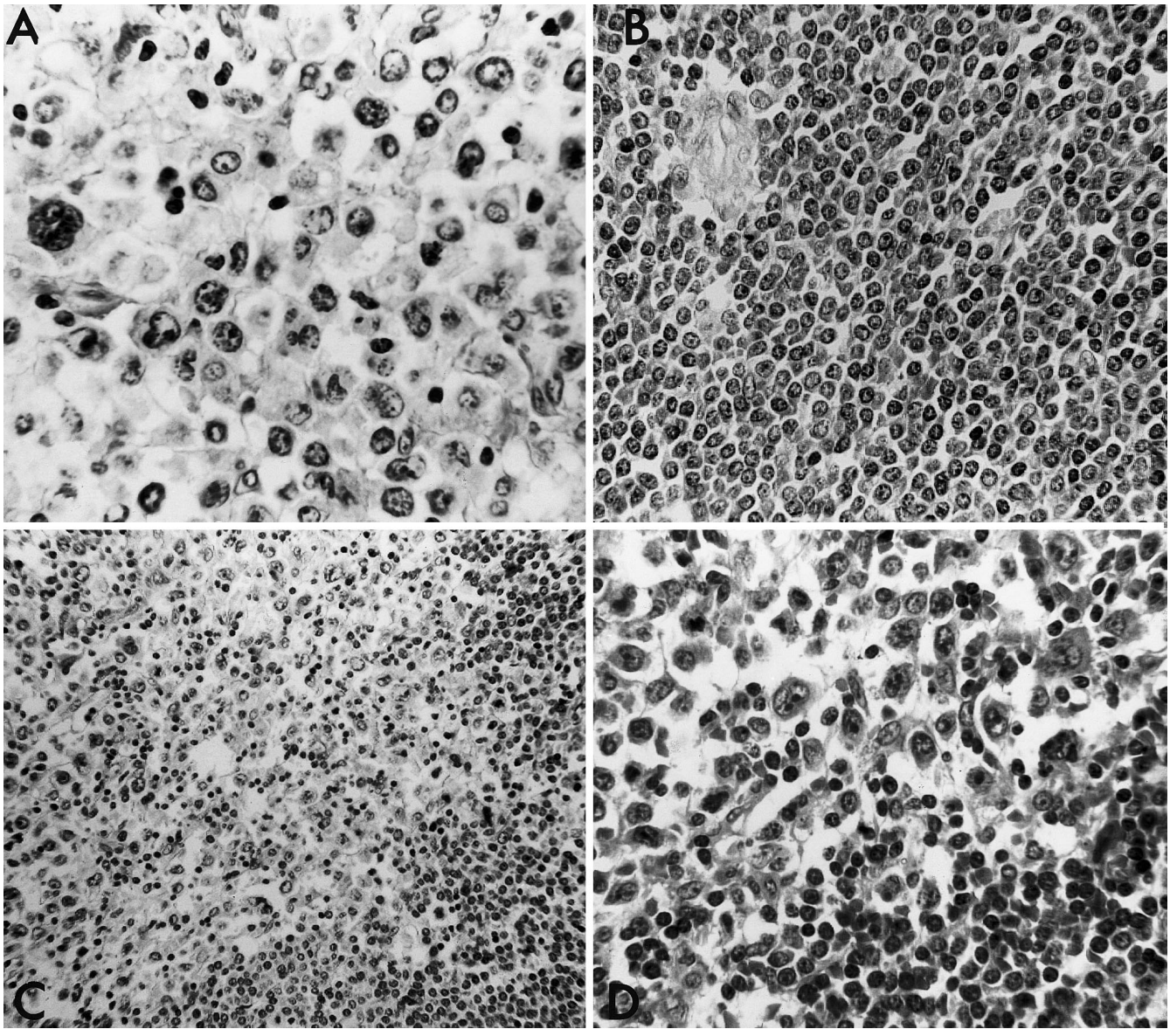


**Fig. 1** **A** The large-cell component of this case of "composite" lymphoma of the spleen is made up of large, pleomorphic lymphoid cells. H&E, ×400. **B** The small-cell component is made up of a somewhat monomorphous population of small lymphocytes, often with evidence of lymphoplasmacytoid differentiation (H&E, ×400). **C, D** Histologically, the border between the large- and the small-cell components is ill defined. H&E, **C** ×200, **D** ×400)

May 1997, leading to a conclusive diagnosis of "composite" lymphoma of the spleen, with lymphoplamacytoid and diffuse large B-cell types. A polychemotherapy (P-VABEC) was started on June 1997. The patient had a recurrence in May 1998, with abdominal lymph node involvement, but no biopsy was performed. A maintenance chemotherapy (Endoxan 100 mg/die) was started, and the patient is at present alive and in partial remission.

## Pathology

The spleen weighed 2,250 g and showed multiple roundish tumour masses up to 12 cm in diameter. The surrounding splenic parenchyma showed an homogeneous appearance, without a clear distinction between the white and the red pulp. The spleen was sampled extensively, and a few representative specimens were snap-frozen in liquid nitrogen and stored at –80°C. Microscopic examination revealed that the grossly detectable lesions were composed of large, pleomorphic lymphoid cells with prominent nucleoli, consistent with a diagnosis of polymorphous large-cell, immunoblastic lymphoma (Fig. 1A). Extensive necrosis was also observed. The surrounding white pulp was involved by a lymphoproliferative disease and was composed of a monotonous population of small lymphocytes, many with evidence of lymphoplasmacytoid differentiation (Fig. 1B). Extensive involvement of the red pulp was also observed. The cytological features and the pattern of involvement were consistent with a diagnosis of non-Hodgkin's lymphoma, lymphoplasmacytoid type (immunocytoma). The border between the two lesions was sharply defined macroscop-

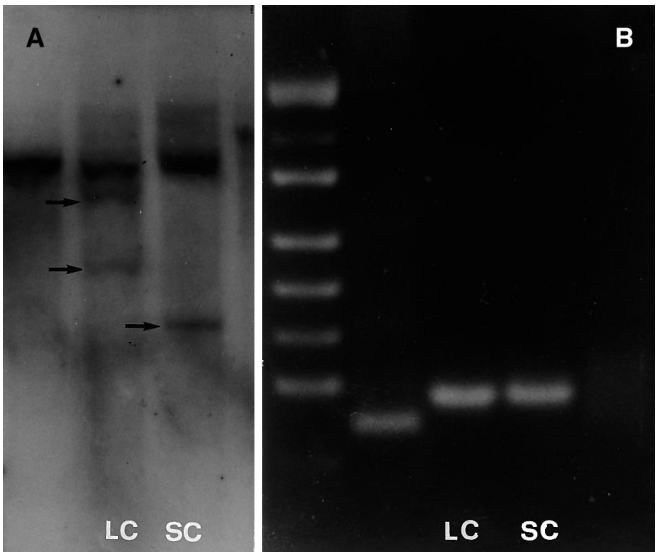


**Fig. 2** **A** Southern blot analysis for IgH status shows two different patterns of clonal rearrangement (*arrows*) in the large-cell (*LC*) and in the small-cell (*SC*) components. **B** PCR analysis for IgH shows two identical rearrangement bands

ically, but somewhat ill defined histologically (Fig. 1C, D).

The immunohistochemical studies were performed on formalin-fixed paraffin-embedded sections as well as on snap-frozen cryostatic sections, by the APAAP method [3]. The immunological profile of the small-cell component was: CD20/CD79a/DBA.44/Bcl2+; IgM+/IgD–; CD25/CD103 –/+ (rare scattered cells); CD3/CD5/CD11c/CD30/CD43/Bcl6–; Ki-67+ cells were <10%. The immunological profile of the large -cell component was: CD20/CD30/CD79a/Bcl2/Bcl6+; CD3/CD5/CD11c/CD43/DBA.44–; Ki-67+ cells were >50%. Furthermore, in both small- and large-cell components a monotypic expression of cytoplasmatic Ig lambda light chains was detected in a few cells. p53 and EBV (LMP-1) were negative in both small- and large-cell components.

Southern blot analysis for IgH configuration was performed on DNA extracted from snap-frozen tissue from both the small- and the large-cell components, using two restriction enzymes (BamHI and Hind III) and a DNA probe for the joining region of IgH (JH) [9]. Clonal rearrangements of the *JH* locus were detected in both DNA samples in the Hind III digest, showing a different pattern of rearrangement (Fig. 2A), whereas no clear clonal rearrangement bands were detected in the BamHI digest. Furthermore, *Bcl*-6 gene status was also studied by Southern blot analysis, which showed a germline configuration in both small- and large-cell components.

PCR analysis for detection of IgH rearrangement was performed on DNA extracted from paraffin-embedded tissue from both small- and large-cell components, using a semi-nested PCR protocol, as previously described [8]. The amplified IgH rearrangement products obtained from the two components were of identical size when visualized on agarose gel electrophoresis (Fig. 2B).

The PCR-amplified fragments were then sequenced using a ABI 373 DNA sequencer, and the analysis showed that the two DNA sequences were virtually identical, with only minimal nucleotide differences (Fig. 3A, B).

## Discussion

The main point of interest of this paper is that this is the first description of a well-characterized case of a composite lymphoma of the spleen in which the two components are a low-grade and a high-grade non-Hodgkin's lymphoma. The macroscopic and histological features were both consistent with this diagnosis, since the two coexistent components had different and distinctive cytomorphological characters, the small-cell component being a lymphoplasmacytoid lymphoma (immunocytoma) and the large-cell component a diffuse large B-cell lymphoma (immunoblastic). The immunological profile was consistent with this diagnosis, showing some minor phenotypical differences between the two lesions. The only one previous report of a "composite" lymphoma of the spleen is that published by Mittal and Nalesnik: in their case the two lesions were Hodgkin's disease and non-Hodgkin's lymphoma [12, 18].

Composite lymphoma is rare and is usually observed in lymph node biopsies, even though extranodal cases have also been reported [1, 16]. The fact that cases of composite lymphoma of the spleen resembling that described in the present report have not been described previously might be related to the low incidence of this condition among lymphomas presenting with prominent splenomegaly.

An additional point of interest in this case, as in all cases of composite lymphoma and similar disorders, such as Richter's syndrome [6, 19], is whether or not the two components have a common clonal origin. This problem requires a multidisciplinary approach using different techniques such as immunohistochemistry, Southern blot hybridization and PCR analysis. Southern blot analysis is usually an effective technique, but it is time consuming and needs to be performed on DNA extracted from frozen tissue, which is not always available in surgical pathology specimens.

In the present case the expression of the same Ig light chain (lambda) in both the small- and large-cell components was suggestive of a common clonal origin, but did not confirm it conclusively. A Southern blot analysis was therefore performed, showing the presence of different patterns of JH clonal rearrangement in the two samples. This finding gave rise to two different hypotheses: that

**Fig. 3** The DNA sequences of the PCR IgH rearrangement products of the large-cell component (**A**) and of the small-cell component (**B**) are shown. The *arrows* indicate the beginning and the end of the amplified sequences. The two nucleotide sequences are virtually identical ▶

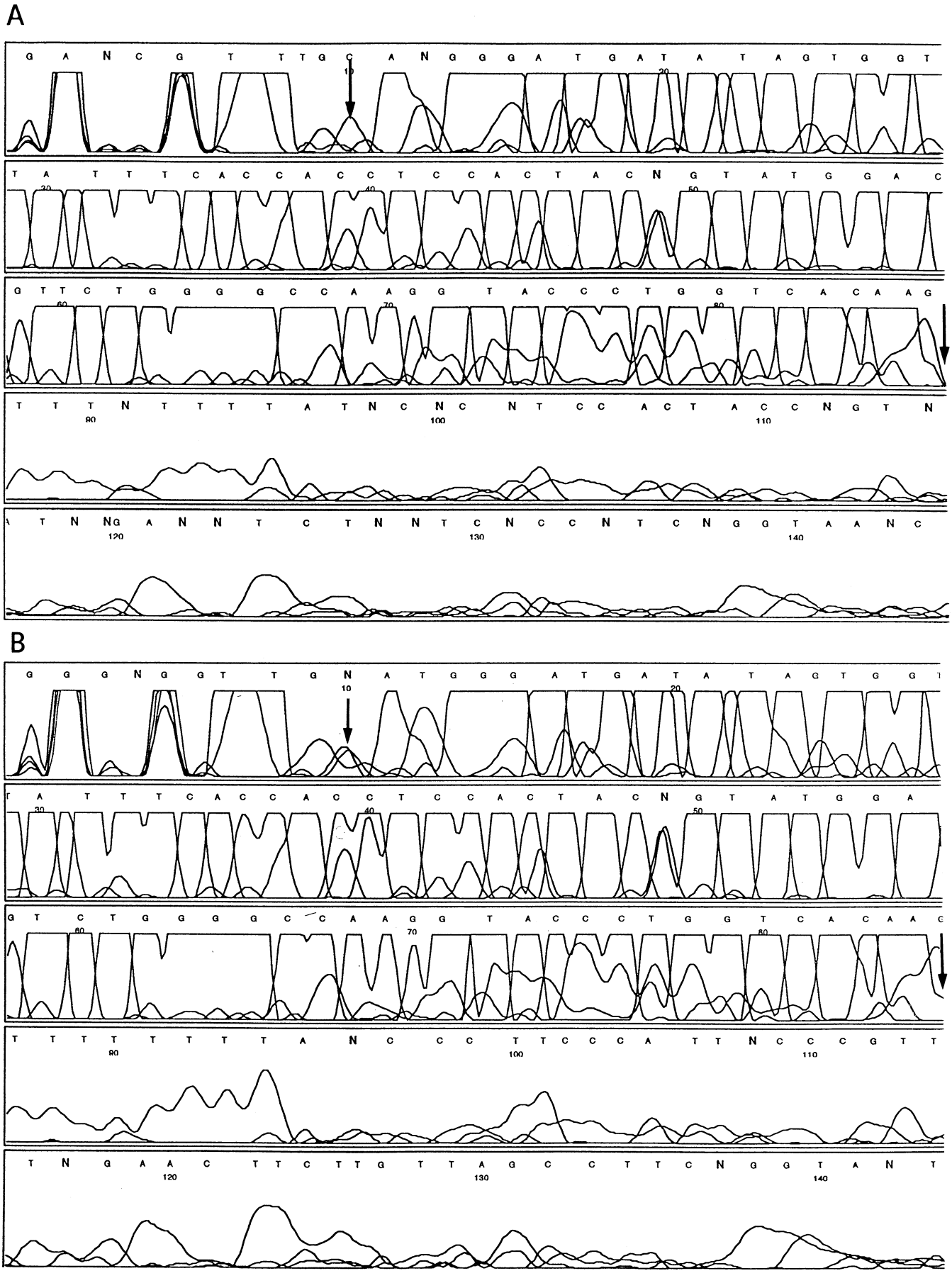


**Fig. 3**

the two components were not clonally related, the different patterns of IgH rearrangements actually reflecting the presence of two distinct clones; or that the different patterns of JH rearrangement did not indicate a different clonal origin, but were due, in the presence of the same clone, to somatic mutations or other post-rearrangement alterations of the *IgH* gene [2, 5]. Since the Southern blot hybridization findings were not conclusive, we performed a PCR analysis, which showed the presence of two identical IgH rearrangement bands, consistent with a monoclonal rearrangement of the *IgH* gene. The PCR-amplified fragments were then sequenced, and the two DNA nucleotide sequences were virtually identical: as a matter of fact, we observed minimal nucleotide differences between the two sequences, which might be due to ongoing somatic mutations or, alternatively, to technical artefacts, similarly to the recent findings by Peng et al. in cases of gastric MALT B-cell lymphoma with low- and high-grade components [21]. Thus, our findings strongly suggest that the two clones were identical and were therefore consistent with a common clonal origin of the two lesions [13].

This case of composite lymphoma of the spleen may be considered a clonal progression (or transformation) of a low-grade B-cell lymphoma to a high-grade B-cell lymphoma, like most cases of Richter's syndrome in chronic lymphocytic leukaemia / small-cell lymphocytic lymphoma [19]. Richter's type transformation has also been reported in occasional cases of lymphoplasmacytoid lymphoma [20]. The findings that LMP-1 and p53 proved to be negative on immunohistochemistry suggest that neither EBV nor p53 played a pathogenetic part in the in the progression of this case, which does not support the recently proposed notion that EBV may be involved in the later stages of clonal progression of non-Hodgkin's lymphoma [7]. This is also a point of difference from some cases of Richter's syndrome [4] and of transformation of follicular lymphoma into diffuse large B-cell lymphoma [17], in which the involvement of p53 mutations has been demonstrated. In conclusion, PCR analysis with direct DNA sequencing of IgH rearrangement products is a useful and effective approach to investigate clonality in cases of "composite" lymphoma and related disorders in which the Southern blot analysis cannot be performed or does not provide conclusive results.

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