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TOPIC HIGHLIGHT

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'Les liaisons dangereuses': Hepatitis C, Rituximab and B-cell non-Hodgkin's lymphomas

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Abstract

Rituximab has provided a revolutionary contribution to the treatment of B-cell non-Hodgkin's lymphomas (NHL). A high prevalence of hepatitis C virus (HCV) infection has been described in B-cell NHL patients. Cases of liver dysfunction in HCV-positive patients have been reported with Rituximab-containing regimens. In this paper we review the recent data regarding the effects of Rituximab in NHL patients with HCV infection. We also added a section devoted to improving communication between oncohaematologists and hepatologists. Furthermore, we propose a common methodological ground to study hepatic toxicity emerging during chemotherapy.

Key words: Rituximab; B-cell non-Hodgkin's lymphoma; Hepatitis C virus; Immunochemotherapy; Methodology

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INTRODUCTION

Rituximab was the first monoclonal antibody approved by the Food and Drugs Administration (November 1997) for the treatment of a human neoplasia: CD20-positive B-cell non-Hodgkin's lymphoma (NHL)[1]. NHL is the most common haematological cancer in adults, and approximately 85% of NHL in adults are of B cell origin.

Hepatitis C virus (HCV) infection is highly prevalent among B-cell NHL patients as compared to controls (15% *vs* 1.5%)[2,3]. Epidemiological studies and meta-analyses indicate that HCV-positive patients have a 2.5-fold increased risk to develop NHL than HCV-negative controls[4]. This figure is highly suggestive of a causative role for HCV infection in the outbreak of lymphomas. The number of B cell NHL attributable to HCV infection varies greatly by country, but can be as high as 10% in highly endemic areas[4,5]. The relative risk of lymphoma development in HCV-positive individuals is similarly increased for all major NHL subtypes and sites of presentation[6]. It has also been shown that HCV-infected patients on inter-

feron therapy who reach a sustained viral response have an hazard ratio of lymphomagenesis significantly lower than untreated patients[7]. Several biological mechanisms linking HCV infection with lymphoma development have been proposed and are still under debate[7,8].

Rituximab is highly selective against CD20+ NHL cells with limited toxic side effects. Nevertheless, untoward reactions have been reported and recently reviewed[1,9]. The reactivation of viral infections is an important adverse event associated with Rituximab administered alone or in combination with chemotherapy (R-CHT). The occurrence of acute hepatitis and even death[10] due to hepatitis B virus (HBV) reactivations in NHL patients treated with R-CHT has been reported since the introduction of Rituximab. Currently these patients are prophylaxed/treated with nucleot(s)ide analogues[10,11]. Data describing the possible role of Rituximab or R-CHT in inducing hepatic toxicity (HT) in HCV-positive B-cell NHL patients have became available only recently.

In this paper we will review the available data regarding this issue and the proposed mechanisms of liver impairment. We also added a section committed to improving communication between oncohaematologists and hepatologists. Furthermore, we propose the basis for a common methodological ground to approach the study of HT emerging during chemotherapy.

SEARCH STRATEGY, STUDY SELECTION CRITERIA AND DATA EXTRACTION

A computerized literature search of MEDLINE was performed using the following search terms: (HCV) AND (Rituximab) AND (Lymphoma), considering English-written literature only. To identify additional studies, the bibliographies of the identified papers were searched for further relevant articles. From the review process were excluded all those studies that (1) described patients not affected with B-cell NHL; (2) from which it was impossible to extract the exact number of NHL patients and/or of HCV-positive patients from heterogeneous series; (3) from which it was not possible to confidently attribute the grade of HT to a specific patient or to a disease group; (4) did not clearly presented the data on HT; and (5) were reported only in abstract form.

STUDIES

Pre-Rituximab era

In the pre-Rituximab era, a few full papers addressed the issue of HCV-positive status as a potential risk factor for the development of liver-related side effects in NHL patients receiving chemotherapy treatments.

In two studies from the far-east, incidence of moderate-severe HT occurred in 18% of HCV-positive B-cell NHL patients treated with standard chemotherapy[12,13]. Conversely, from the available data in the HCV-negative group the incidence of moderate-severe HT ranged from 0% to 14%[12-14]. According to the Takai paper, this difference was not statistically significant[13].

Contrasting data were obtained in a French study conducted on a large population of patients with diffuse large B-cell NHL (DLBCL). Chemotherapy induced the emergence of moderate-severe HT events in 12/23 HCV-positive patients[15]. This percentage (52%) was larger than that observed previously[16], and in addition HCV-positive status was shown to have a negative effect on overall survival (OS, $P = 0.02$), but not on event-free survival. Development of HT determined treatment modification in 47% of HCV positive patients. However, in this study more aggressive chemotherapy regimens were admittedly adopted. No association between initial severity of hepatic disease and subsequent development of HT was described. In the papers previously examined, data on HCV-RNA trends were not systematically collected or reported, thus hampering the identification of an unambiguous relationship between HCV replicative activity and HT development.

Thus, in the pre-Rituximab era no clear indications had emerged to define the HCV-positive B-cell NHL a group at higher risk of developing HT as the result of standard chemotherapy. The occurrence of severe HT was considered to be so rare as to deserve the publication of case reports[17].

Into the Rituximab era

In the early B-cell NHL Rituximab trials, HCV-positive status was not an exclusion criteria for treatment, and no HT[18], or only mild elevation of liver enzymes had been described[15]. In addition, the trials did not report the HCV-status of the patients. At any rate, no data had emerged suggesting a possible role for HCV-positive status as a risk factor for HT during Rituximab treatment.

Initial experiences with R-CHT in HCV-positive B-cell NHL were published at the beginning of the new millennium. Emergence of HT was rarely reported in earlier case reports or case series, and HCV-RNA levels, not consistently determined, did not show uniform trends as the result of Rituximab treatment[19-26]. A large retrospective study performed in Italy on a group of consecutive HCV-positive patients with DLBCL, showed that only 15% (20/132) of patients developed moderate-severe HT, reconfirming the low incidence reported in previous studies[25]. Occurrence of HT determined a modification of the scheduled therapy: dose reduction/prolongation of intervals between chemotherapy cycles in case of grade 2-3 HT (11%), treatment interruption in the most severe cases (4%, grade 3-4 HT). In this study Rituximab was combined with standard chemotherapy protocols in 26.5% of patients (35/132). None of the Rituximab-treated patients developed moderate-severe HT, and only five developed a mild liver enzymes increase not requiring treatment discontinuation. No data on HCV-RNA trends were provided. Progression free survival (PFS) and OS at 5 years were respectively 51% and 72%. Unfortunately, lack of a control group did not allow for comparison of

clinical outcome with HCV-negative patients. However, results suggested that Rituximab might be combined safely with chemotherapy in HCV-positive NHL patients, but careful monitoring of liver function and viremia was recommended[27].

In 2008, Ennishi published an interesting paper dealing with HCV-positive B-cell NHL patients from Japan treated with Rituximab; HCV genotype was characterized, and HCV-RNA was tested monthly during and after chemotherapy[28]. Serum transaminases, albumin and total bilirubin were also monitored. Five of the six anti-HCV antibody-positive patients were HCV-RNA positive at baseline. An increase of HCV-RNA (ranging 0.73-1.06 $\log_{10}$) during treatment was observed in all these 5 patients. However, only one patient, genotype 2a, developed severe HT. In this patient HCV-RNA decreased below the limit of detection at the time of maximal serum transaminases peak, to increase again after R-CHT was stopped. The authors were, however, unable to explain this phenomenon and why in the remaining 4 patients, despite an increase of HCV-RNA during Rituximab treatment, there was no evidence of HT[28]. Recently, a letter from Italy also suggested that genotype 2 might represent a specific risk factor for the development of HT in NHL patients treated with Rituximab-containing regimens[29]. However, data were limited and it was not clear if pre-treatment HCV-RNA were available for all patients, and not all patients developing an increase in HCV-RNA developed HT.

In 2010, another retrospective study from Italy reported the data on 160 HCV-positive patients with NHL[30]. In this paper HCV-positive patients were carefully studied for liver and haematological disease status, and chemotherapy treatment used. The common terminology criteria for adverse events (CTCAE) were used to define HT, with a non-standardized adaptation for patients with elevated transaminases at baseline[31]. HCV genotype was available in 60 of the 146 HCV-RNA positive patients (41%). Significant HT occurred in 24 patients (15%), and 8 (5%) did not complete the planned treatment because of it. Five (18%) of the 28 patients treated with R-CHT developed HT: 3 stopped therapy, while the other 2 had to postpone it. Nine of 132 (7%) patients treated with Rituximab-free regimens developed HT. Thus, even if barely missed, HT incidence was however not significantly different between the two groups ($P = 0.07$), even if limitations due to sample size cannot be excluded. HCV-RNA quantification did not correlate with ALT levels, and was thus defined not useful to predict the occurrence of HT. Severe HT developed more frequently in genotype 1 patients (26%), than among genotype 2 (3%), with 85% of moderate-severe HT events developing in the former group ($P = 0.02$). Maximum increase of HCV-RNA over the baseline levels was more frequently reported among genotype 1 patients than genotype 2 ($P = 0.05$). Five years OS was significantly lower in patients who had developed significant HT (62% *vs* 84%, $P = 0.006$). Also median PFS was shorter for patients developing HT (2 years *vs* 3.7 years, $P = 0.03$). However, lymphoma relapse and progression were related to a previous episode of significant HT only in aggressive NHL subtypes ($P = 0.01$). The authors concluded that Rituximab use is related to a slightly higher occurrence of toxicity that does not circumvent its use in HCV-positive NHL. They however underlined that occurrence of HT in HCV-positive NHL patients caused a significant limitation in the delivery of an effective immunochemotherapy[31].

In 2010, we published a retrospective study on a group of Italian patients with CD20-positive, B-cell NHL treated with Rituximab-CHT[32]. Nine patients (8.6%) were HCV positive and viremic at baseline. Two were also HBsAg-positive but HBV-DNA-negative at baseline and received appropriate prophylaxis[10], remaining HBV-DNA negative thereafter. Three (33%) of the 9 HCV-positive patients and none of the 95 negative developed HT ($P < 0.001$). All had normal ALT before treatment. In two, ALT peak developed approximately 5 mo after the end of treatment. One of them, also in this case a genotype 2a, developed icteric hepatitis (total bilirubin 7.8 mg/dL) without relevant prothrombin time alteration during the acute phase. The remaining patient developed HT while on treatment, but chemotherapy was not stopped, and he completed the full course of treatment. In the patients developing HT, HCV-RNA did not follow the ALT trend, with two patients showing increases and one a decrease over baseline. No significant correlation was detected between ALT and HCV-RNA levels before, during, and 12 mo after HT development. Only one patient had advanced liver fibrosis. At the 12-mo follow-up, no liver-related death or complication had developed in HCV-positive patients developing HT, ALT had decreased but not regressed to normal, and patients were alive and in remission for their haematological disease. We concluded proposing HCV-positive status as a risk factor for the development of HT in B-cell NHL patients receiving Rituximab-containing regimens[32].

By the end of 2010, Ennishi published the results of the currently largest, multicenter retrospective study on HT and the prognosis of patients with DLBCL treated with Rituximab-containing regimens[33]. They analyzed 553 patients: 131 HCV-positive and 422 HCV-negative. HCV-positive patients were significantly older, had more advanced disease (higher international prognostic index, > 1 extranodal site and spleen involvement) than those HCV-negative. Thirty-six (27%) HCV-positive and 13 (3%) HCV-negative patients developed severe HT ($P < 0.0001$). HT determined dose modification in 12% and chemotherapy withdrawal in 4.6% of HCV-positive patients. Six HCV-positive patients died of hepatic failure, caused by hepatocellular carcinoma in four. Similar to our results, HCV infection was confirmed as a significant risk factor for the development of severe HT at multivariate analysis (hazard ratio: 14.72, 95% CI: 6.37-34.03, $P < 0.001$). Increased pre-treatment transaminases levels were predictive for the development of severe HT. However advanced haematological disease stage, and not HCV-

positive status or development of HT, affected negatively PFS and OS. Dose delays and liver failure associated with severe HT development were the likely explanations for the non-significant trend toward the reduced late survival (> 2 years) observed after therapy ($P = 0.07$)[34], and it could be speculated that if a larger sample size would have been available, this difference in late survival among HCV-positive patients might have been significant. HCV-RNA levels (collected before, during treatment, 1 mo after treatment and 2-6 mo after having stopped treatment) increased significantly during immunochemotherapy ($P = 0.006$). However, complete data at all the 3 time points were available only for 26% of patients, and median HCV-RNA increase on treatment was of 0.5 $\log_{10}$ only. It was also not clear if the HCV-RNA increase did at all correlate with ALT levels and HT events. No data on genotypes were available[35,36]. Ennishi's data are indeed stimulating, but to clarify the possible role of viremia in determining liver injury, further studies are needed to prospectively evaluate viral load variations and parameters of liver damage in Rituximab-treated patients developing and not developing HF before, during and after therapy.

POSSIBLE MECHANISMS OF HT DEVELOPMENT IN B-CELL NHL PATIENTS TREATED WITH RITUXIMAB-CONTAINING REGIMENS

Some insights on HT development in B-cell NHL patients treated with Rituximab can be derived from the literature. The spontaneous occurrence of hepatitis flares in HCV patients have been described. Rumi *et al*[37] observed that in a group of HCV patients, genotype 2c and 1b, followed for 71 mo, the incidence of hepatitis flare was more frequent among the former group as compared to the latter (31% *vs* 7.5%), and that these episodes correlated with fibrosis progression[37]. Such observations may suggest that a similar phenomenon could be occurring in HCV-positive NHL patients, a speculation further supported by papers suggestive of a specific role for genotype 2 in determining the development of HT in these patients[24,29,32]. However, spontaneous fluctuations of HCV-RNA in chronic infection, usually of limited magnitude (maximum 1 $\log_{10}$), have been described[38]. Interestingly, when we transformed to $\log_{10}$ the available HCV-RNA data from the above listed studies, the delta over basal differences observed were mostly comprised within this range or slightly over it (data not shown). However, the data by Rumi were derived from immunocompetent HCV-infected patients, and thus differences in study design and population do not allow either for direct comparison or to conclude that HT occurring in Rituximab-treated patients are part of the natural history of HCV infection. As far as genotype is concerned, more data are needed to establish a major role for genotype 2 in determining HT in Rituximab-treated HCV-positive NHL patients.

It is a widely accepted concept that immunosuppressive treatment might determine an increase in hepatitis virus replication, leading to an expansion of infected hepatocytes. Following treatment interruption and immunoreconstitution infected hepatocytes are lysed. This mechanism holds true for hepatitis B infection[39,40], but has not yet been demonstrated for HCV infection. Since these viruses markedly differ in their virological characteristics and in their immune escape and survival strategies[41], these hypotheses need to be tested and verified. For instance, after years in which it was widely believed that glucocorticosteroids enhanced HCV-RNA replication, it has instead been recently demonstrated *in vitro* that these drugs actually reduce it, increasing instead HCV entry into hepatocytes[42]. Similar data on Rituximab are however not available.

According to another widely cited letter, Rituximab treatment could affect viral replication inducing a decrease of immunoglobulin of the M class[43]. In chronic infection, HCV circulates usually bound to IgM, so its decrease, secondary to Rituximab treatment, might determine a loss of immune control over the virus allowing for an increase of HCV-RNA. Again, HCV-RNA increase has not been clearly associated with the development of HT. In addition, this report regarded a patient with HCV-related cryoglobulinemia, and modifications of viral load were determined during concomitant treatment with pegylated interferon, further hampering data interpretation.

Direct drug-related liver toxicity[44], or development of Rituximab-immunomediated phenomena[9,45,46] should also be considered as possible alternative causative mechanisms. Rituximab might induce HT per se, with HCV infection representing a risk factor for its development, as reported for antiretroviral drugs[47].

Even if intriguing and reasonable, the currently available data do not provide evidence to support any of the above speculations.

EVALUATION OF HT

In order to supply a common background between oncohaematologists and hepatologists, to better communicate and manage our patients, we felt it opportune to develop a section commited to the evaluation of hepatotoxicity.

BASELINE EVALUATION

When performing baseline evaluation of oncohematological patients scheduled to undergo chemotherapy, a search for liver disease and viral hepatitis infection is recommended. Patient evaluation and history-taking should address relevant issues such as previous history/diagnosis of liver disease, drug or alcohol abuse, previous blood transfusion, use of prescription and non-prescription drugs, herbal remedies[48]. Signs and symptoms of liver disease should also be searched for.

Table 1 Clinical significance of commonly performed biochemical liver tests

Liver test	Clinical significance
AST, ALT	Cytolysis
Total bilirubin, γGT, ALP	Cholestasis
Prothrombin time/INR, Albumin, pCHE	Synthesis
Decreased platelet number	Portal hypertension

AST: Aspartate-aminotransferase; ALT: Alanine aminotransferase; γGT: γ-glutamyl transpeptidase; ALP: Alkaline phosphatase; INR: International normalized ratio; pCHE: Pseudo-cholinesterase.

Successively, evaluation of serum enzymes, even with its well known limitations[49,50], is commonly employed. Using these tests we tentatively explore and categorize the possible presence of signs of liver dysfunction. A list of the most commonly performed biochemical liver function tests and the function they explore/express is provided in Table 1.

Presence of chronic infection with hepatitis viruses should be investigated by testing for viral serum markers: antibodies to HCV, hepatitis B surface antigen, and hepatitis B core antigen, and hepatitis B surface antigen. HBV-DNA testing is performed to differentiate active from inactive carriers. In the case of hepatitis B, management guidelines for oncohaematological, and immunosuppressed patients have been proposed[10], while no defined strategies have been provided for HCV-positive oncohaematological patients. Patients positive for HCV-antibodies undergo qualitative HCV-RNA testing to verify the presence of active infection. Order of magnitude of viral replication and genotyping are not clinically relevant, unless an antiviral treatment is scheduled[38]. Routinely testing HCV-RNA levels during oncohematological treatment remains a debated issue, and considering its cost, it should preferably be done in a controlled clinical study setting until relevant data can support its use in clinical practice.

Imaging studies are part of the baseline staging of haematological disease. These can also provide important information to detect signs of underlying cirrhotic liver disease. A list of the information provided by liver ultrasound, the most commonly performed imaging test, is summarized in Table 2. Similar information can also be provided by computed tomography and magnetic resonance.

Hepatic biopsy, with its intrinsic limitations and risks[51], is still regarded as the gold standard to define the extent of liver damage. It can provide additional information, such as hepatic involvement secondary to the haematological disease, and is used to confirm the presence of cirrhosis, but it is rarely performed in this setting given the priorities and reduced times imposed by the need of treatment typical of oncohematological diseases.

Transient eleastography (Fibroscan®) is useful to determinate non-invasively and with sufficient accuracy the presence/absence of liver cirrhosis in HCV patients[52]. However its use in oncohematological patients has never been studied in detail.

Table 2 Liver ultrasound: information for the management of liver diseases

Parenchymal signs	
Dimensions	Hepatomegaly
	Caudate lobe hypertrophy
	Quadrate lobe hypotrophy
Echo pattern	Coarse: typical of liver fibrosis
	Coarse nodular: micronodular cirrhosis
	Attenuation sign/bright liver: typical of hepatic steatosis
Nodules	Benign *vs* malignant lesions
	Skip areas (fatty liver)
Surface	Nodular *vs* smooth
Extraparenchymal signs	
Indirect signs of portal hypertension	Splenomegaly
	Ascites
	Collateral vessels
	Lack of splenic and/or superior mesenteric vein diameter variations during breathing
	Increased portal vein diameter
Doppler ultrasound	Inversion/reduction of portal vein flow
	Portal vein thrombosis/cavernomatosis

If cirrhosis has been diagnosed, its severity should be defined. Several scoring systems have been validated, and the most used are the Child-Pugh-Turcotte (CPT), and the model of end stage liver disease[53-55]. The former is based on the evaluation of 3 biochemical, i.e., albumin, bilirubin and prothrombin time/international normalized ratio (PT and INR respectively), and two clinical variables (ascites and portal-systemic encephalopathy), while the latter also requires creatinine levels in addition to bilirubin and INR. Even if limitations for chemotherapy administration are suggested only for CPT stage "C" cirrhotic patients[50], stratification by grade of impaired liver function may provide an additional tool to estimate disease burden at baseline and during follow up.

ON-TREATMENT MONITORING

Patients undergoing chemotherapy are followed up to estimate the effects of treatment on disease course. Physical examination, laboratory tests, and imaging studies concur to the early detection of side effects. However, not only chemotherapy can cause HT. In case of alterations to liver biochemistry, use of drugs other than chemotherapy, especially antibiotics, novel hepatitis virus infection or reactivation, and possible liver involvement by haematological disease progression should be considered and ruled out.

In case HT is caused by chemotherapy, events should be described and categorized by standardized grading systems. Oncohaematologists usually adopt the US National Cancer Institute CTCAE[49,50]. Toxic effects grades are scored 1 to 5. The CTCAE also provides descriptors for definite hepatic events (i.e., liver dysfunction, viral hepatitis), but these definitions are composite, more complex and not easy to adopt. By definition, when serum transaminases [aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT), respectively], alkaline phospatase, or γ-glutamyl transpeptidase increase > 5-20

Table 3 Patterns of drug induced liver disease according to laboratory tests

Pattern	ALT	ALP	ALT/ALP
Hepatitis pattern	⩾ 3 ULN	--	⩾ 5 ULN
Cholestatic pattern	--	⩾ 2 ULN	⩽ 2 ULN
Mixed pattern	> 3 ULN	> 2 ULN	> 2 ULN to < 5 ULN

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ULN: Upper limit of normal. Adapted from Verma *et al*[56].

Table 4 Mild, moderate and marked elevations of serum liver enzymes

Enzyme	Mild	Moderate	Marked
sAT	> 2-3	2, 3-20	> 20
ALP	< 1.5.2	1.5-5	> 5
γGT	> 2-3	2-3-10	> 10

Numbers are times (X) upper limit of normal value. sAT: Serum aminotransferases; ALP: Alkaline phosphatase; γGT: γ-glutamiltranspeptidase. Adapted from Ahmed *et al*[57].

× upper normal level (ULN), the adverse event is defined as grade 3 and categorized as "severe", while increases > 20 × ULN are grade 4 and are defined as "life threatening or disabling". It is thus a descriptive terminology trying to express a clinical interpretation with a numeric grade. However, sometimes patients with these toxicities can be asymptomatic.

In a more hepatological perspective, severity of HT is commonly derived from the biochemical pattern determined by drug exposure (Table 3)[56]. Patients with the hepatocellular pattern can be asymptomatic or report fatigue and right upper quadrant pain. Serum transaminases increment can be variable, but all patients with clinical or laboratory evidence of moderate/severe acute hepatitis (Table 4)[57] should have immediate measurement of PT/INR, serum bilirubin and careful evaluation for subtle alterations in mentation to exclude the presence of acute liver failure (ALF)[58]. While the degree to which transaminases are elevated does not adequately mirror liver impairment, jaundice instead represents a good predictor of mortality in drug-related liver injury. Bilirubin persistently > 3 × ULN (biliary obstruction and Gilbert's syndrome having been ruled out), is burdened with a 10% mortality (range, 5%-50%)[56]. When INR ⩾ 1.5), and there is evidence of an altered sensorium, the diagnosis of ALF is established. Hospital admission in this setting is mandatory[58]. Extrapolating these data to the oncology setting is difficult, but as a general rule any drug associated with increase of serum AST/ALT > 3 × ULN should be stopped if jaundice has developed[59].

The cholestatic pattern can mimic biliary obstruction or the course can be more indolent with jaundice and pruritus. Mortality appears to be less than in the hepatocellular pattern (1%-7.8%), and death is usually not liver-related[56]. The mixed pattern has probably the lowest mortality (around 2%)[60,61].

CONCLUSION

HCV-positive status seems to represent a risk factor for the development of HT in patients with B-cell NHL treated with Rituximab. The degree of possible HT is variable ranging from moderate to severe. However, larger prospective studies, designed with a strong methodological basis and using standard descriptive terminologies for HT are warranted. These studies should properly define hepatological events and are needed to clarify the causative relationship, and to uncover toxicity mechanisms. Until then, HCV-positive patients receiving Rituximab should be carefully followed up to rapidly detect and properly manage the possible development of liver-related side-effects.

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