2- Research Proposal Summary	**2- خلاصة مقترح البحث**
Summary (Not more than 150 words)	الخلاصة بما لا يزيد عن 150 كلمة:-

Patients with end-stage liver disease (ESLD) presenting with hyper-dynamic shock to the intensive care unit have high mortality reaching 70%. Adrenal dysfunction is being recognized recently as an important entity in patients presenting with septic shock. Considering the similarities between septic shock, shock-related to adrenal insufficiency and hyper-dynamic shock in cirrhotic patients, we hypothesize that adrenal dysfunction is present in some cirrhotics with shock and that steroid replacement may reduce the hemodynamic instability and possibly improve outcome. This study is designed as prospective randomized controlled trial of steroid vs. placebo in cirrhotic patients presenting with hyper-dynamic shock. ACTH stimulation test, cytokine levels (TNF-a, IL-6) and procalcitonin levels will be obtained to correlate the hemodynamic response with biochemical markers. If hydrocortisone replacement proved to be beneficial, it will be of a great value to the Saudi patients with ESLD

Abbreviations: ACTH=Adrenocorticotropic hormone, ESLD =End Stage Liver Disease, ICU=Intensive Care Unit, PCT =procalcitonin, ESR=erythrocyte Sedimentation rate, CRP =C-reactive protein, TNF=tumor necrosis factor, IL = Interleukin

Keywords : shock , sepsis , liver failure , cirrhosis , adrenal insufficiency, procalcitonin.

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3- Literature review

- A brief summary about topic of research proposal and its importance
- A comprehensive and updated literature review

3- المسح الأدبي

-نبذة عن موضوع مقترح البحث وأهميته
-مسح شامل وحديث للأدبيات

End–stage liver disease secondary to cirrhosis represents a major public health challenge. In the United States, chronic liver disease is the tenth common cause of death in adults [1]. In the Kingdom of Saudi Arabia, cirrhosis is also a major cause of morbidity and mortality. We reported from our Intensive Care Unit (ICU) at King Abdulaziz Medical City (KAMC) that complications related to end-stage liver disease were the underlying causes for 12% of all admissions and 29% of all deaths[2]. We have examined the outcome of cirrhotic patients admitted to the ICU and found the ICU mortality to be 57% and hospital mortality 74% [3]. Figure 1 shows our statistics for the last three years and illustrates the significance of this public health problem. These alarming figures occur despite the aggressive medical management, the use of state of art technologies and the high resource utilization by these patients.

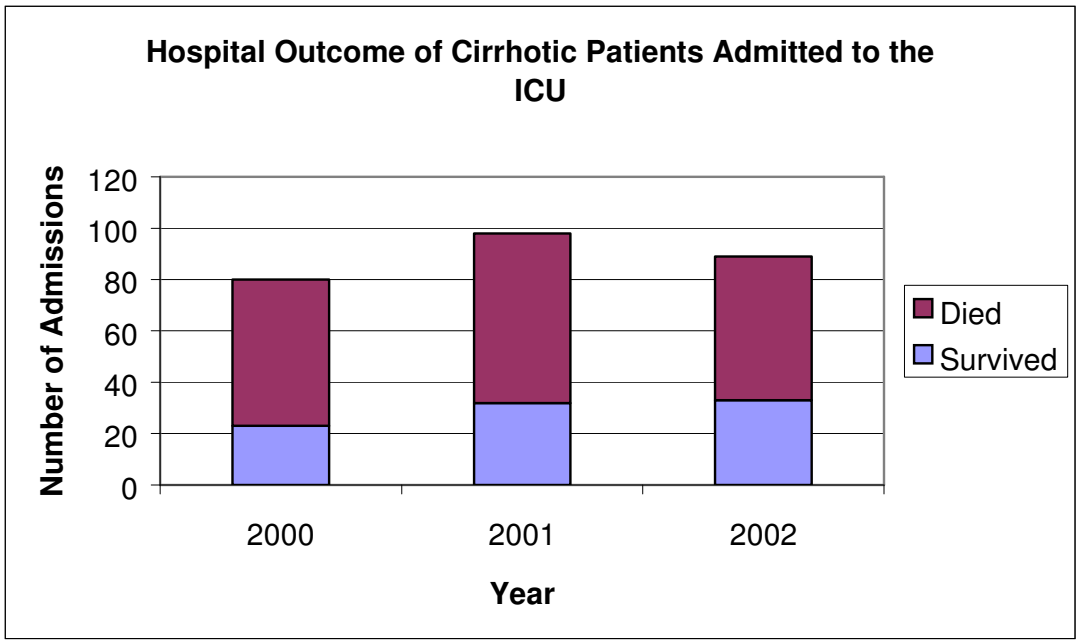

Figure 1: Hospital outcome of cirrhotic patients admitted to the ICU at KAMC.

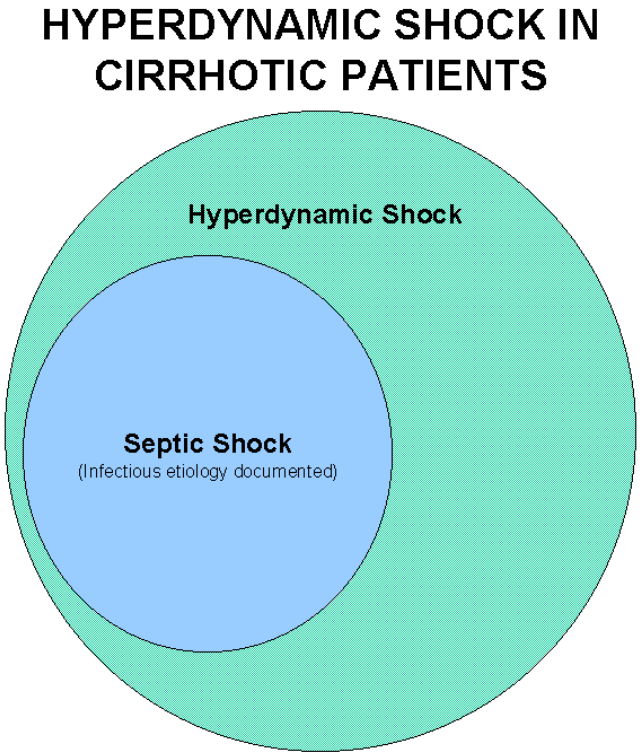

Figure 2: Hyperdynamic shock in cirrhotic patients.

Patients with end-stage liver disease often present to intensive care unit with a distributive shock picture characterized by low systemic vascular resistance and hyperdynamic cardiac function [4]. While sepsis is responsible for significant proportion of these cases, an infectious etiology often cannot be demonstrated. Figure 2 represents our current established understanding of hyperdynamic shock in cirrhotic patients. Since adrenal crisis state shares similar features, it is possible that some of those patients with liver failure have a component of adrenal dysfunction. Figure 3 represents this hypothesis. Such concept would have profound clinical implications. Adrenal insufficiency is a treatable condition with steroid replacement. Such treatment would be expected to improve the hemodynamic instability, reduce vasopressor requirements and potentially improve organ dysfunction and reduce mortality. Such effects have been clearly demonstrated in septic shock patients [5]. It has never been studied specifically in cirrhotic patients and partially in those with hyperdynamic shock without documented infection. To our knowledge, there is only one study that assessed the prevalence of adrenal insufficiency in acute liver failure patients [6]. In that study, no steroid

replacement was given. Therefore, our study tests a novel concept and can improve the outcome of this unfortunate group of patients with high mortality. Considering the prevalence of cirrhosis in Saudi Arabia and the poor outcome, such therapy will have a very positive and a welcomed impact.

D. BACKGROUND INFORMATION ***(add pages if necessary as explained under the detailed instruction but keep content concise. Number additional pages)***

The following literature review will cover the following areas: (1) Adrenal dysfunction in septic shock, (2) Adrenal dysfunction in cirrhotic patients, (3) Cytokines and adrenal dysfunction and (4) The utility of procalcitonin in differentiating septic from non- septic shock.

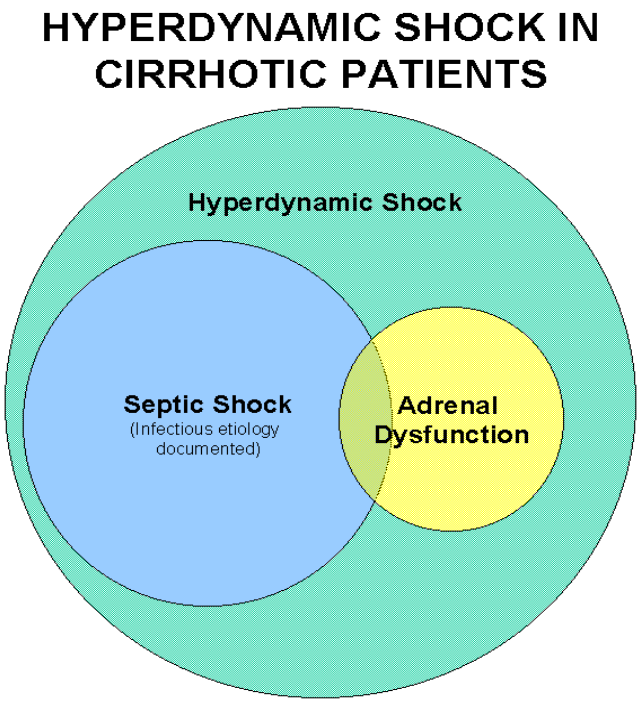

Figure 3: Adrenal dysfunction may be a player in shock state among cirrhotic patients.

ADRENAL DYSFUCNTION IN SHOCK STATES

It is firmly believed that the hypothalamic –pituitary-adrenal axis is recruited early in septic states to counter regulate the inflammation through the synthesis and secretion of cortisol – the stress hormone. Cortisol secretion is increased in response to stress states such as infection, surgery and trauma to extremely high levels reaching up to 6 folds. This increase is associated with loss of the physiologic diurnal variation of secretion [7, 8, 9]. Several mechanisms have been implicated in the pathogenesis of adrenal insufficiency in patients with septic shock. It has been shown that tumor necrosis factor (TNF ∝) is a potent inhibitor of ACTH-induced cortisol production and steroidogenic P450 enzyme gene expression in cultured fetal adrenal cells [10]. Brunetti, *et. al.* showed that interleuki1 i β activates the hypothalmo-pituitary-adrenal axis and that lipopolysuccharid (LPS) inhibits basal and IL--β -induced ACTH release [11]. These studies and others support the notion that adrenal insufficiency in sepsis may be of several types: primary and secondary. Soni, *et.al.* found that 24% of his patients with septic shock had adrenal insufficiency. ACTH levels were lower than control and so were the levels of IL-6. They suggested a link between reduced IL-6 levels and the understimulation of the pituitary adrenal axis [12].

The subnormal adrenal response to inflammatory stress is termed functional or relative hypoadrenalism to differentiate it from the classic primary hypoadrenalism seen in Addison's disease. Diagnosing this state can be a hard task. It is often based on clinical suspicion when the patient remains hypotensive despite adequate fluid resuscitation. Cortisol levels measurements can be inaccurate and rather misleading. There is no normal ranges for random cortisol levels, rather a consensus amongst the scientists in this field. Most investigators take a cutoff of 15-20 µg per deciliter as acceptable [13]. Due to the variation of random cortisol levels according to age, gender and physiologic states, a stimulation test was recommended to evaluate the functional status of adrenal glands. After obtaining baseline level for cortisol, cosyntropin –a synthetic ACTH – is given followed by measurement of cortisol levels after 30 and 60 minutes. Despite the controversy surrounding this test, it is widely accepted that an increment below 9 µg per deciliter [250 nmol per liter] from the base-line cortisol level to the highest cortisol level confirms adrenal dysfunction and is associated with higher mortality [14, 15, 16].

Hormonal replacement in shock states especially septic shock had its ups and downs over the last few decades [17,18]. Initial approaches with short term high dose steroids (up to 40 grams) proved no benefit but rather more harm and high doses of steroids have been abandoned since the1980s[18.]. Based on new evidence, hormonal replacement resurfaced. Annane and coworkers [5], conducted a randomized controlled trial on 300 patients with septic shock who were randomly assigned to receive either hydrocortisone (50-mg intravenous bolus every 6 hours) and fludrocortisone (50-µg tablet once daily) (n = 151) or matching placebos (n = 149) for 7 days. The study proved lower mortality for the corticosteroid group and faster reversal of shock. Similar results have been shown in other studies that used low dose replacement of corticosteroids [19, 20, 21.] In most of these studies levels of cortisol and response to ACTH stimulation tests were linked to survival from septic shock [22]. There has been unparalleled excitement over the last few years about the physiologic replacement of hormones in patients with sepsis ad septic shock. Promising results have been already published with insulin, corticosteroids and vasopressin.

ADRENAL DYSFUCNTION IN LIVER CIRRHOSIS

Based on the strong similarities between septic shock and liver failure states, Harry and coworkers studied the potential adrenal dysfunction in acute liver failure patients using the ACTH stimulation test [6]. In this study 45 patients with acute hepatic failure were tested and 62% of them found to have abnormal test .The investigators concluded that adrenal dysfunction is quite common in patients with acute hepatic failure. However, the investigators did not examine whether steroid replacement will be beneficial.

Adrenal insufficiency in liver failure patients can be caused by several mechanisms. One of the postulated explanations is that the adrenal response is at its maximum which will lead to less increment on the ACTH stimulation test. This was shown in a study by Moran and colleagues who found an inverse linear relationship between the baseline cortisol and the increments [23]. They also found that higher mortality was associated with adrenocortical hypo-responsiveness [23]. Harry and coworkers did not find any correlation between adrenal function and coagulation profiles making adrenal hemorrhage unlikely as a cause for the dysfunction noted in their study [6].

We have previously demonstrated high mortality for patients with cirrhosis once they are admitted to intensive care units [3]. We hypothesize that our patient's population might have an endogenous adrenal dysfunction besides their liver failure state rendering them resistant to vasoactive medications. Proving associated adrenal dysfunction may have an impact on morbidity and mortality especially that it is potentially reversible with exogenous corticosteroids supplementation as the current literature suggest.

CYTOKINES AND ADRENAL FUNCTION IN CIRRHOTICS WITH SHOCK

Data on cytokine effects on adrenal function in cirrhotics with hyperdynamic shock is extrapolated mainly from the data on septic patients considering the similarities .

Human Interleukin 6 (IL-6) is a 184 amino acid polypeptide produced by various cells, including T- and B-cells, monocytes,fibroblasts, keratinocytes, endothelial cells, mesangial cells, astrocytes, bone marrow stroma cells and several tumor cells. It regulates the growth and differentiation of various cell types with major activities on the immune system, and inflammation. These multiple actions are integrated within a complex cytokine network. Although most normal controls have undetectable levels of IL-6 in their serum, huge quantities of IL-6 are detected in patients with septic shock [24,25]. Along with IL-1 and TNF, it induces the synthesis of acute phase proteins (APP) by hepatocytes, each cytokine or combination of cytokines showing a preferential pattern of APP production. IL-6 induces ACTH production [26]. The elevation of serum IL-6 precedes that of acute phase proteins and may thus be a sensitive early parameter to investigate inflammatory conditions [27,28].

Several studies demonstrated that inteleuken-6 deficient animals have reduced adrenal response and increased mortality after introduction of systemic infection. This is similar to what was found in humans [29,30] . Soni reported that septic patients with adrenal dysfunction have lower IL-6 than those who did not [12].

Tumor Necrosis Factor Alpha (TNF-∝) also named cachectin, is a 157 amino acid unglycosylated polypeptide cytokine mainly produced by activated macrophages. Lipopolysaccharide (LPS), the cell wall component of gram-negative bacteria (endotoxin), is a potent stimulus for TNF-∝ production by macrophages and TNF-∝ is an important mediator of the well-known in vivo effects of LPS such as fever, shock and activation of neutrophils. It has been shown that tumor necrosis factor (TNF ∝) is a potent inhibitor of ACTH-induced cortisol production and steroidogenic P450 enzyme gene expression in cultured fetal adrenal cells [10.]

INFECTION VERSUS NO INFECTION: ROLE OF PROCALCITONIN

Since the prompt and early diagnosis of sepsis is highly important, it is essential to differentiate between infectious and non- infectious processes. Because of the pre-existing hyperdynamic state in cirrhotic patients, this differentiation based on clinical grounds can be very difficult. The use of microbiologic data such as blood cultures is helpful in only fraction of patients. In septic shock trials, only 30-40% of patients with documented septic shock have positive blood cultures [31]. Imaging studies are often not successful in identifying the source of infection or they can lead to false positive results which engage the physicians in a vicious cycle of unnecessary tests and procedures. These difficulties in establishing the diagnosis of sepsis set a challenge for the clinicians and press for a more accurate and sensitive test in this area. Biochemical markers have gained lately growing interest to aid in the diagnosis of sepsis. Some of them have been found non- specific (ESR, CRP: C-reactive protein). Tumor necrosis factor (TNF) - α, IL-1β, IL-6 and IL-8, have been studied with variable results[32]. Serum procalcitonin (PCT) has emerged recently as a marker to help in the differentiation of sepsis.

Procalcitonin, a, 116-amino-acid protein is the precursor of calcitonin. Plasma levels are low (below 0.1 ng/ml) but rise considerably in bacterial infections [33]. Numerous studies have shown that procalcitonin (PCT) has some useful diagnostic utility in differentiating severe inflammatory conditions, in particular, cases of sepsis and septic shock [34,35,36]. Moreover, the PCT can also be utilized to monitor critically ill patients and the course of therapy [37-41].

Balci and coworkers [42] studied the accuracy of PCT in comparison to other biochemical markers (C-reactive protein, IL-2, IL-6, IL-8 and tumor necrosis factor-α).on 33 critically ill patients diagnosed with SIRS (systemic inflammatory response syndrome), Sepsis and septic shock. They found that PCT exhibited the greatest sensitivity (85%) and specificity (91%) as well as positive and negative predictive values in differentiating patients with SIRS from those with sepsis. Some reports suggested that if the PCT value is less than 5 ng/ml, an alternative diagnosis to sepsis should be entertained such as adrenal insufficiency [43]

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4- Objectives

4- الأهداف

Research proposal objectives and expected results

أهداف مقترح البحث ونتائجه المتوقعة

1) To evaluate the adrenal function in cirrhotic patients admitted to the intensive care unit with non- hemorrhagic shock.
2) To assess the effect of low dose hydrocortisone in patients with end-stage liver disease with hyperdynamic shock.
3) To correlate between the clinical effect and the responsiveness to ACTH stimulation test.
4) To examine the cytokine responses in these patients and then correlate that with the diagnosis of adrenal dysfunction.
5) To assess the value of procalcitonin in differentiating infectious and non-infectious etiologies. In particular, whether low procalcitonin level (5 ng/ml) predicts the presence of adrenal dysfunction.

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Appendix to: Arabi YM, Aljumah A, Dabbagh O, et al. Low-dose hydrocortisone in patients with cirrhosis and septic shock: a randomized controlled trial. *CMAJ* 2010. DOI:10.1503/cmaj.090707.

5- Utilization	5- الاستفادة من المشروع
Contribution of research results in addressing development issues in the Kingdom.	- كيفية الإستفادة من النتائج البحثية في معالجة قضايا التنمية في المملكة

IMPLICATIONS/ UTILIZATION OF THE STUDY RESULTS

As indicated before, cirrhosis is a significant health problem in Saudi Arabia and carries very poor prognosis when present with shock to the Intensive Care Units. In a previous paper comparing our patients receiving liver transplants with similar group from the University of Wisconsin, USA, we found that our patients were significantly different [44]. Hepatitis C is responsible fro the majority if cirrhotic cases compared to alcoholic liver disease and our patients were significantly sicker as estimated by APACHE II scores. These findings support the need for local data about this extremely important group.

The main implications of our study can be summarized as follows:

A. Improving the outcome of these critically ill patients.

B. Improving our understanding of the pathophysiology of shock in this group of patients.

C. Providing new tools, such procalcitonin to help in differentiating infectious vs. non-infectious causes of shock so more focused therapies can be given.

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6- Methods and Procedures

6- خطة العمل

Techniques of accomplishing the research proposal objectives. كيفية تحقيق أهداف مقترح البحث

Patients:

Cirrhotic patients admitted to the Intensive Care Unit with non-hemorrhagic shock.

EXCLUSION CRITERIA:

- Pure hypovolemia
- Hemorrhagic shock
- Known adrenal insufficiency
- Prior steroid use
- DNR order
- Patients in terminal condition
- Contraindication to steroids
- Refused consent

Design:

- Randomized control trial

Primary End Points:

- 28 day mortality

Secondary End Points:

- ICU and hospital mortality
- Duration of vasopressor requirement
- The correlation between the clinical response, the results of ACTH stimulation test and the cytokine levels.
- The predictive value of procalcitonin and adrenal dysfunction.
- ICU Length of stay
- Duration of mechanical ventilation
- Reversal of shock (MAP>65 for 24 hrs without pressors)
- GI bleeding

- Hyperglycemia
- Nosocomial infections as per NISS
- Arrythemias, requiring treatment.
- Requirement and duration of vasoactive medications

Procedure:

A. Consent. (*Please see attached form).*

B. Baseline data. (*Please refer to the attached data collection form*).

Baseline data collection includes demographics, cirrhosis etiology data, the source microbiology of sepsis, Acute Physiology and Chronic Health Evaluation (APACHE) II Score, Sequential Organ Failure Assessment (SOFA) score. In addition, the following laboratory data will be collected (usually performed routinely in the ICU): Hemoglobin, Platelet count, blood glucose, International Normalized Ration (INR), AST, ALT, Alkaline Phosphatase, Billirubin, GGT, Lactic Acid, and Ammonia. Also we will document Etomidate use.

C. ACTH Stimulation Test

- Draw baseline ACTH and Cortisol
- Give 250 mcg of ACTH (Cosyntropin)
- Draw cortisol level at 30 and 60 minutes

D. Randomization

Randomization will be performed centrally (in the Pharmacy). Medications (Intravenous hydrocortisone 50 mg vs. Placebo every 6 hours) will be assigned using randomization table. The study will be double blinded.

E. Daily data collection. *(Please refer to data collection forms).*

The following will include vasopressor requirements and SOFA scores, as indicators of organ failure and daily fluid balance. The patient status at discharge from ICU and hospital and at 28 days of randomization will be documented.

F. Sample size

There are no studies examining the effect of hydrocortisone on outcome in cirrhotic patientsBased on our previous study, we estimated 28-day mortality to be 90%. We anticipated 20% Absolute Risk Reduction (ARR) and 22% relative risk reduction (RRR) with hydrocortisone therapy. In Annane et al. study on general ICU septic patients, 28-day mortality was reduced from 63% to 53% for all patients (10% ARR and 16% RRR. As such, we needed 75 patients in each group to using a two-sided type-I error of 5% and power of 80%. Interim analysis is planned at 75 patients.

G. Cytokine Assays

Both TNF-α and IL-6 will be assayed using ELISA kits. A solid phase Enzyme Amplified Sensitivity Immunoassay (EASIA) performed on microtiter plate. The assay is based on an oligoclonal system in which a blend of monoclonal antibodies (MAbs) directed against distinct epitopes of TNF-α or IL-6 will be used. Standards or samples (serum) containing TNF-α or IL-6 react with capture monoclonal antibodies (MAbs 1) coated on the microtiter well. After incubation, the occasional excess of antigen is removed by washing. Mab 2, the horseradish peroxidase (HRP)-labelled-antibody, is then added. After an incubation period allowing the formation of a sandwich: coated MAbs 1 – TNF- α or IL-6 - MAb 2 - HRP, the microtiter plate is washed to remove unbound enzyme labeled antibodies. Bound enzyme-labeled antibodies are

measured through a chromogenic reaction. Chromogenic Solution (TMB+H2O2) is added and incubated. The reaction is stopped with the addition of Stop Solution (H2SO4) and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance which is proportional to the TNF- α concentration. A standard curve is plotted and TNF- α concentrations in a sample is determined by interpolation from the standard curve. The use of the EASIA Reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in high sensitivity in the low range and in an extended standard range.

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- Cited References

8- قائمة المراجع

ينبغي للمراجع أن تكون مرتبة حسب ورودها في البحث

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