1	Second line tests in the differential diagnosis of neoplastic and non-neoplastic hypercortisolism: a
2	systematic review and meta-analysis
3	Authors: Alessandro Mondin ^{1,2} , Mattia Barbot ^{1,2,3} , Giacomo Voltan ^{1,2} , Irene Tizianel ^{1,2} , Carlotta Keiko
4	Vedolin ^{1,2} , Pierluigi Mazzeo ^{1,2} , Martina Lazzara ^{1,2} , Marco Boscaro ² , Carla Scaroni ^{1,2} , Filippo Ceccato ^{1,2,3}
5	¹ Department of Medicine DIMED, University of Padova, Padova, Italy
6	² Endocrine Disease Unit, University-Hospital of Padova, Padova, Italy
7 8	³ Department of Neuroscience DNS, University of Padova, Padova, Italy
9	
10	Supplementary data
11	

- Supplementary table 1. Protocol for the application of QUADAS-2 tool. Every item is evaluated for risk of bias and
 applicability concern, except the latter: flow and timing item was assessed only for the risk of bias domain.

1) Patient selection			
Risk of bias	Applicability concern		
Low risk of bias was attributed to complete and	Low applicability concern was attributed to studies		
consecutive series without post-hoc inappropriate	including only patients presenting clear pCS-related		
exclusion of patients. Concerns regarded random or	conditions in the control group. Concerns rose when the		
incomplete inclusions or inappropriate exclusions of	study also included CS-excluded patients in the NNH/pCS		
patients. In the presence of both the concerns above, the	group. When the number of CS-excluded patients		
study was deemed at high risk of bias.	exceeded that of patients presenting pCS-related		
	conditions the applicability concern was deemed high.		
2) Index test			
Risk of bias	Applicability concern		
Studies prospectively designed with predefined	Researchers evaluated whether the index tests (and their		
threshold presented a low risk of bias. Some concerns	protocol and interpretation) matched the review		
were addressed for ROC-based thresholds or in case of a	question and provided the grading accordingly (low/		
retrospective design. High risk of bias was addressed for	some concerns/ high).		
retrospective studies using a ROC-based threshold.			
3) Reference standard			
Risk of bias	Applicability concern		
Low risk of bias was assessed for studies providing	Low concern was addressed for studies including only CD		
histological confirmation of CS or in case of	patients presenting mild to moderate hypercortisolism.		
hypercortisolism remission after surgery. Some concerns	In case of studies including non-pituitary CS (i.e., EAS,		
rose for studies including patients with persistence after	ACES) and/or CD patients with severe hypercortisolism		
surgery and without histological confirmation. High risk	researchers could rise some concerns or decide for a high		
of bias was attributed to studies with CS diagnosis based	concern judgement.		
on progressing clinical or biochemical features.			
4) Flow and timing			
Risk of bias			
Low risk of bias was assigned to studies with at least one year of follow-up to define NNH/pCS patients and with			
index tests applied prior to reference standard for CS patients. Some concerns rose in case of CS patients receiving			
the index tests after the reference standard and/or in case of short follow-up for defining NNH/pCS (i.e., < 1 year). If			
these patients were the majority of the population studied, a high risk of bias was attributed to the study.			

Supplementary figure 1. Pooled effects for sensitivity of Dex-CRH test (a), Desmopressin test (b) and CRH test (c). CS =
 Cushing's syndrome; Dex = dexamethasone; CRH = corticotropin realising hormone; CI = confidence interval.



- 20 Supplementary figure 2. Pooled effect for specificity of Dex-CRH test (a), Desmopressin test (b) and CRH test (c).
- 21 NNH/pCS = non-neoplastic hypercortisolism/pseudo-Cushing; Dex = dexamethasone; CRH = corticotropin realising hormone; CI = confidence interval.
- 22

а Author, Year Non responders NNH/pCS Proportion 95%-CI Yanovski JA, 1993 19 19 [0.82; 1.00] 1.00 21 12 Martin NM, 2006 24 0.88 [0.68: 0.97] Gatta B, 2007 14 0.86 [0.57; 0.98] . Erickson D, 2007 29 30 0.97 [0.83; 1.00] Pecori Giraldi F, 2007 20 23 0.87 [0.66; 0.97] Batista D, 2008 20 21 0.95 [0.76; 1.00] Reimondo G, 2008 14 15 0.93 [0.68; 1.00] Valassi E, 2009 39 46 0.85 [0.71; 0.94] . Alwani RA, 2014 19 19 1.00 [0.82; 1.00] Random effects model 211 0.92 [0.84; 0.96] Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0.0991$, p = 0.880.6 0.7 8.0 0.9 1 Dex CRH Specificity b Author, Year Non responders NNH/pCS Proportion 95%-CI Moro M, 2000 0.97 [0.83; 1.00] 29 30 Pecori Giraldi F, 2007 19 21 0.90 [0.70; 0.99] Tirabassi G, 2010 26 28 [0.76; 0.99] 0.93 0.95 [0.85; 0.99] Rollin G, 2015 53 56 Random effects model 0.94 [0.83; 0.98] 135 Heterogeneity: $I^2 = 0\%$, $\tau^2 = 0$, p = 0.820.7 0.75 0.8 0.85 0.9 0.95 Desmopressin Specificity С Author, Year Non responders NNH/pCS Proportion 95%-CI Yanovski JA, 1993 19 19 1.00 [0.82; 1.00] Tirabassi G, 2011 30 1.00 [0.88; 1.00] 30 Ceccato F, 2020 20 31 0.65 [0.45; 0.81] 80 0.99 [0.00; 1.00] Random effects model Г Heterogeneity: $I^2 = 0\%$, $\tau^2 = 11.1962$, p = 1.000.2 0.4 0.6 0.8 1 **CRH Specificity**

Supplementary figure 3. Funnel plot analysis for Dex-CRH test (a), Desmopressin test (b) and CRH test (c). Dex = dexamethasone; CRH = corticotropin realising hormone.

