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ORIGINAL PAPER

Cosyntropin-stimulated salivary cortisol in hospitalized patients with hypoproteinemia

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Abstract Analysis of adrenocortical function in acutely ill, hospitalized patients can be challenging due to changes in plasma binding proteins. This study used dynamic testing of salivary cortisol levels to evaluate adrenal function in hospitalized patients with low/low-normal plasma protein concentration in whom adrenal insufficiency was suspected. Twenty-eight patients with low serum albumin and proteins hospitalized for acute illness were evaluated for decreased adrenocortical function because of clinical presentations suspicious for adrenal insufficiency. Baseline and post cosyntropin-stimulated levels of serum total and salivary cortisol levels were assessed. Data were gathered by a retrospective analysis of medical records. Eight patients had normal peak serum total and salivary cortisol responses, consistent with intact adrenocortical function. Five patients had abnormal peak serum total and salivary cortisol responses indicating decreased adrenocortical function. Fifteen patients had subnormal peak serum total cortisol, but normal peak salivary cortisol responses indicating normal adrenal function. Salivary cortisol testing can identify hospitalized patients with apparently intact adrenal function in whom low serum protein confounded interpretation of serum total cortisol measurements. Salivary cortisol is a clinically useful surrogate for serum free cortisol in dynamic testing of adrenocortical function.

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Introduction

Evaluation of adrenocortical function in hospitalized patients has traditionally been performed with dynamic testing using synthetic $ACTH_{(1–24)}$ (cosyntropin) stimulation of serum total cortisol [1]. Most total serum cortisol is protein-bound, and only 5–10% of total cortisol is free (unbound) and available to exert biological effects [2, 3]. Hypoproteinemia can confound interpretation of these results because cortisol circulates bound to plasma cortisol binding globulin (CBG) (aka transcortin) and albumin [4]. In severe acute illness, CBG levels are decreased [3, 5]. Furthermore, in prolonged states of malnutrition and chronic illness, levels of albumin and CBG are low [5, 6].

Optimum testing for adrenal function involves the measurement of the bioactive component of circulating glucocorticoids [4]. However, the use of serum free cortisol testing is not currently feasible in acute clinical situations with hospitalized patients [7]. Serum free cortisol is not a standard measurement in most laboratories. In specialty labs where it is performed, serum free cortisol testing is time consuming and labor intensive. It has a long turnaround time, so results are usually not available in a timely enough manner to guide acute clinical decision-making for the treatment of hospitalized patients.

Salivary cortisol can be used as a surrogate for serum free cortisol [8–10]. Salivary cortisol is in equilibrium with plasma free cortisol [2, 11] and is independent of salivary flow rate [11, 12]. Measurements of salivary cortisol have a high degree of correlation with serum free cortisol levels as measured by equilibrium dialysis [8], even in critically ill

patients [13]. Salivary cortisol also correlates extremely well with total serum cortisol when plasma proteins are normal [14]. Salivary cortisol testing can be performed in standard hospital laboratories with results available with a turn-around time of <3 h [15]. With these rapid results, salivary cortisol testing can be used to guide clinical decisions in acutely ill hospitalized patients.

The salivary cortisol response to stimulation with cosyntropin has proved useful in the evaluation of patients with critical illness [13], chronic renal failure and end-stage renal disease [16, 17], HIV infection [18], adrenal insufficiency [19, 20], and pregnancy and patients treated with oral birth control pills and hormone replacement therapy [6, 21–23]. The purpose of the present study is to evaluate the use of cosyntropin-stimulated salivary cortisol in acutely ill hospitalized patients suspected of having adrenal insufficiency, but with the confounding factor of low serum albumin [13].

Results

Table 1 shows the characteristics of the patients subdivided into three groups as shown in Table 2. Group 1 consisted of

Table 1 Patient characteristics grouped by serum and salivary cortisol responses to cosyntropin

Patient	Age	Sex	Chief complaint	Prior steroid use?[a]	Steroid treatment after test?	Survivor at 1 month?
Group 1: Serum cortisol normal, salivary cortisol normal n = 8						
3	80	F	Hypotension	D,H	Yes	Yes
4	78	M	Subdural hematoma Surgery	No	No	Yes
5	65	F	Mitral valve repair surgery	E,P	No	No
17	69	M	Megace use	M	No	Yes
22	54	F	Hypotensive episode	No	No	Yes
23	78	M	Weakness, weight loss	No	Yes	Yes
25	58	M	Hypotension, weight loss	D	No	Yes
27	48	F	Hypoglycemia, hyponatremia	No	No	Yes
Group 2: Serum cortisol abnormal, salivary cortisol abnormal n = 5						
6	40	F	Orthostasis, palpitations	No	Yes	Yes
7	75	M	Urosepsis	M	Yes	Yes
11	66	M	Orthostasis, hyponatremia	D	Yes	Yes
12	61	F	Hypotension, empty sella	No	Yes	Yes
18	56	M	Hypotension, hyponatremia	D	Yes	Yes
Group 3: Serum cortisol abnormal, salivary cortisol normal n = 15						
1	49	M	Hypoglycemia	No	No	Yes
2	39	F	Orthostasis	H	Yes	Yes
8	53	M	Hypotension, syncope	No	Yes	No
9	85	M	Weakness, weight loss	D	Yes	Yes
10	77	F	Steroid cessation after taper	P	No	Yes
13	84	F	Hypotension, hyponatremia	H,M	Yes	No
14	59	M	Orthostasis, weakness	No	No	Yes
15	40	F	Hypotension, hyponatremia	H	Yes	Yes
16	44	M	Hypotension, pneumonia	P	Yes	Yes
19	69	M	Hypotension, hyponatremia	P,M	No	Yes
20	83	M	Hyponatremia, CABG surgery	P	No	Yes
21	57	F	Hypotension, wound infection	No	Yes	Yes
24	69	M	Orthostasis, neutropenic with wound infection	D	No	No
26	80	M	Hyponatremia	No	Yes	Yes
28	46	M	Orthostasis, syncope	No	No	Yes

[a] Prior steroids defined as oral or parenteral physiological hydrocortisone (H) therapy (stopped >12 h before testing to prevent interference with the cortisol assay), epidural steroid injections (E) within 2 months, dexamethasone (D) either before or during testing, megestrol acetate (Megace; M) therapy, or prednisone (P; stopped >24 h before testing to prevent interference with the cortisol assay). Patient numbers reflect the chronological order in which patients were studied

Table 2 Plasma ACTH, serum albumin, protein, and cortisol, and salivary cortisol measurements

Patient	Albumin (g/dl)	Total protein (g/dl)	Basal serum cortisol (nmol/l)	Stimulated serum cortisol (nmol/l)	Basal salivary cortisol (nmol/l)	Stimulated salivary cortisol (nmol/l)
*Group 1: Peak serum and salivary cortisol **normal** n = 8*						
3†	<1	2.9	598	522	121.0	160.0
4	3.1	5.9	466	632	24.7	125.0
5†	2.3	4.7	262	726	57.4	68.8
17	2.2	5.7	232	569	10.2	69.7
22†	1.4	4.3	475	602	43.8	37.6
23	2.8	6.4	417	618	25.6	21.0
25	2.5	5.3	494	723	10.3	49.5
27	3.6	7.0	419	897	4.9	132.0
Mean (SEM)	2.4 (0.3)	5.3 (0.5)	420 (43)	661 (42)	37.2 (13.5)	83.0 (17.7)
*Group 2: Peak serum and salivary cortisol **abnormal** n = 5*						
6	3.9	6.5	22	293	1.6	4.8
7	1.8	4.8	207	304	5.2	6.8
11	2.7	4.6	177	370	2.2	9.8
12	2.5	4.6	359	389	14.7	16.7
18	2.8	6.3	30	395	1.2	4.8
Mean (SEM)	2.7 (0.3)	5.4 (0.4)	159* (62)	350* (22)	5.0* (2.5)	8.6* (2.2)
*Group 3: Peak serum cortisol **abnormal**, peak salivary cortisol **normal** n = 15*						
1	1.2	3.9	240	442	13.3	35.9
2	2.7	5.6	83	251	7.1	23.8
8†	2.6	6.6	406	414	50.7	43.2
9	2.8	4.5	99	428	20.5	40.1
10	3.4	4.7	177	491	4.9	39.5
13	2.0	4.2	284	441	19.7	42.7
14	3.5	6.9	290	356	21.8	39.8
15	1.9	5.3	293	378	100.0	90.8
16	2.2	5.0	215	447	5.7	21.0
19†	1.5	3.9	135	405	40.5	82.0
20†	2.5	5.7	251	419	13.4	44.2
21†	2.1	5.1	212	406	15.4	53.0
24†	1.7	4.5	458	392	20.3	15.6
26	2.1	5.6	102	447	3.3	22.0
28	3.1	6.1	174	417	3.5	26.9
Mean (SEM)	2.4 (0.2)	5.2 (0.2)	228* (28)	409* (14)	22.7 (6.5)	41.4* (5.5)

* Indicates different from Group 1 ($P < 0.05$)

† Indicates patients who received 250 mcg cosyntropin; all others received 5 mcg

Normal cosyntropin-stimulated serum cortisol >497 nmol/l (18 mcg/dl)

Normal cosyntropin-stimulated salivary cortisol >18 nmol/l (0.65 mcg/dl)

Normal serum albumin 3.5–5.0 g/dl

Normal serum protein 6.5–8.0 g/dl

Patient 24 was assigned to group 3 because the basal salivary cortisol was >18.0 nmol/l

8 patients who had normal test results for both serum total cortisol and salivary cortisol responses to cosyntropin. These patients were deemed to have intact adrenal function. Group 2 had 5 patients with subnormal results for both serum total cortisol and salivary cortisol suggesting biochemical adrenal insufficiency. Group 3 had 15 patients with normal peak salivary cortisol suggesting intact adrenal function, but subnormal peak serum total cortisol. There were no differences in the proportion of patients who had received prior steroid therapy in Group 1 (4 of 8) versus

Group 2 (3 of 5), versus Group 3 (9 of 15). Furthermore, there was no difference in the number of days from admission to salivary cortisol testing between the groups (medians [25–75% CI] for Group 1 = 8 days [5–12], Group 2 = 9 days [5–26], and Group 3 = 13 days [5–20]).

There were no differences in serum albumin or total protein between the three groups (Table 2). *Basal* serum cortisol in Group 2 and Group 3 (both with subnormal *peak* serum cortisol) was significantly less than Group 1 (normal *peak* serum cortisol response to cosyntropin). *Basal* salivary cortisol was lower in Group 2 (subnormal *peak* salivary cortisol response to cosyntropin) compared to Group 1 (normal *peak* salivary cortisol response to cosyntropin). *Basal* salivary cortisol in Group 3 tended to be lower than Group 1 although they were not significantly different. Of 28 patients, 16 had received prior or concurrent corticosteroid therapy that was the initial motivation for testing adrenal function due to concern for HPA axis suppression. Steroids that interfere with the cortisol assays (prednisone and hydrocortisone) were discontinued at least 12 h before sampling. We emphasize that this was not to allow recovery of the hypothalamic–pituitary–adrenal axis, but was only to avoid interference of exogenous steroids with the serum and salivary cortisol assays.

Table 2 and Fig. 1 shows individual and mean (±SEM) peak serum and salivary cortisol concentrations. Although the peak salivary cortisol levels for Group 3 exceeded the threshold for normal values, the mean peak salivary cortisol for Group 3 was significantly less than that of Group 1, and significantly greater than that of Group 2. In Group 1, three of the patients were stimulated with 250 mcg of cosyntropin, while four patients were stimulated with 5 mcg of cosyntropin. In Group 2, all patients were stimulated with 5 mcg of cosyntropin. In Group 3, five patients were stimulated with 250 mcg of cosyntropin, while ten patients were stimulated with 5 mcg of cosyntropin. There was no significant difference in the peak serum cortisol with the different cosyntropin doses used within Groups 1 and 3.

Of 15, 7 patients in Group 3 were not treated with glucocorticoids following testing, thereby avoiding the potentially detrimental effects of unnecessary glucocorticoid exposure in the setting of acute illness. Eight subjects in Group 3 did subsequently receive glucocorticoid therapy after testing despite normal peak salivary cortisol responses based on the subnormal peak serum cortisol responses. The attending physicians opted to administer corticosteroid therapy to two patients in Group 1 despite normal cortisol responses to cosyntropin because of hypotension. In both cases, hypotension resolved and corticosteroid therapy was discontinued. Although this study was not designed to assess outcomes, there did not appear to be a difference in 1-month survival of Group 3 patients with or without glucocorticoid therapy (Table 1). Based on both abnormal peak serum and salivary cortisol responses to cosyntropin, Group 2 patients were considered to have biochemical adrenal insufficiency. All of the Group 2 patients were treated with corticosteroids and were survivors at 1 month.

Discussion

This study identified hospitalized patients with presumably normal adrenocortical responses to cosyntropin when

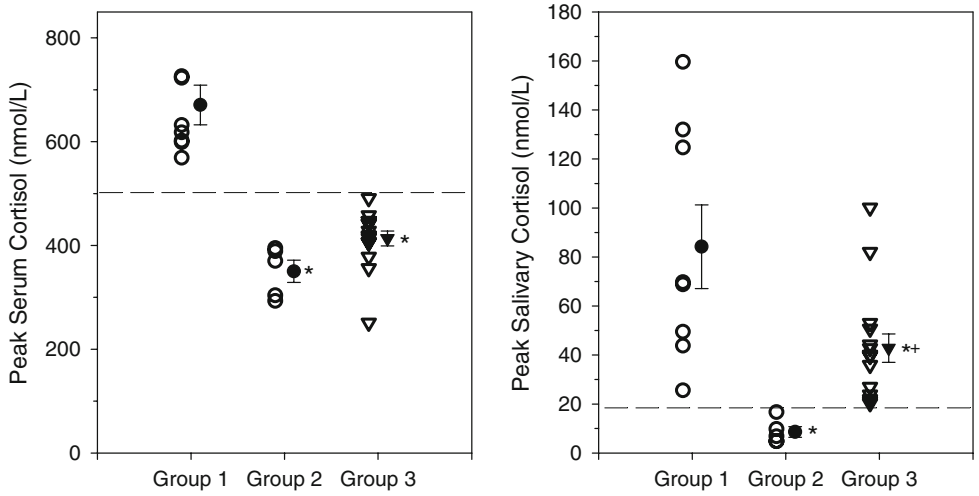

Fig. 1 Peak serum and salivary cortisol responses to cosyntropin in hospitalized patients. Group 1 had normal serum and salivary cortisol responses; Group 2 had abnormal serum and salivary cortisol responses; Group 3 had discordant results with subnormal serum, but normal salivary cortisol responses. * Indicates different from Group 1; + indicates different from group 2 ($P < 0.05$). The horizontal line is the normal peak serum cortisol (>497 nmol/l [18.0 mcg/dl]) and salivary cortisol (>18.0 nmol/l [0.65 mcg/dl]) in response to cosyntropin regardless of time of day or baseline cortisol

assessed using salivary cortisol despite attenuated serum cortisol responses probably due, at least in part, to decreased plasma binding proteins. Dynamic testing of adrenal function is confounded by hypoproteinemia because the low levels of serum proteins may result in a decrease in serum total cortisol concentration [3, 4, 6, 24]. As a result, some patients may be unnecessarily treated with glucocorticoids and exposed to the potential adverse effects of high-dose steroids (hyperglycemia, myopathy, neurotoxicity, fluid retention, immunosuppression, and suppression of endogenous glucocorticoid secretion). The optimal treatment plan would be one based on measurements of only the bioactive glucocorticoid levels. This can be done with measurement of serum free cortisol [4, 6, 13]. However, use of serum free cortisol is not clinically practical due to the fact that the test results usually cannot be obtained in a short enough time to guide clinical decision-making in acutely ill hospitalized patients.

Other factors can confound testing with serum total cortisol results. Estrogen-containing medications and high-estrogen conditions (such as pregnancy) result in high CBG concentrations, which will elevate serum total cortisol concentration without truly representing an increased serum free cortisol level [6, 23]. Heterophile antibodies present in the serum can interfere with serum total cortisol immunoassay testing and result in false low measurement values [25].

Our study demonstrates the utility of salivary cortisol testing for dynamic assessment of adrenal function in hospitalized patients. The test results were performed in a typical clinical scenario using on site standard laboratory equipment. Most important is the fact that salivary cortisol accurately reflects serum free cortisol and is thus an assessment of the biologically active, circulating cortisol activity [12].

The test results differentiated the patients into three groups. Groups 1 and 2 both had concordant results for testing with both serum total cortisol and salivary cortisol. Group 1 clearly had intact adrenal function and Group 2 clearly had biochemical hypoadrenalism. Group 3 had discrepant results for peak serum total cortisol and peak salivary cortisol testing, with serum total cortisol levels indicating subnormal adrenocortical function, while salivary cortisol levels showed normal adrenocortical function.

While Group 3 had peak salivary cortisol levels over the threshold for normal values, the mean peak salivary cortisol for Group 3 was significantly less than that of Group 1, and significantly greater than that of Group 2. This suggests that Group 3 has diminished but still intact adrenal function in combination with low protein levels as the dual root cause of the low serum total cortisol levels. This was not likely due to prior steroid use since this was similar in frequency between the groups.

The patients with normal adrenal function by both serum and salivary cortisol (Group 1) managed to mount a normal peak serum cortisol response to cosyntropin, despite low serum albumin and protein levels. The reasons for this could be that the actual steroidogenic response to cosyntropin was higher than normal as has been suggested previously [4] and that this overcame any decrease in serum binding proteins to yield a "normal" serum cortisol level. It is also possible that CBG levels were normal despite lower albumin and protein levels in serum. The measurement of CBG is a reference laboratory test and does not have a fast enough turn-around time to be clinically useful in patients with acute illness. Hence, salivary cortisol has been shown to be a useful clinical surrogate for the measurement of serum free cortisol or CBG [13].

It is not clear that Group 3 truly had intact adrenal function. Our reference range established for salivary cortisol with cosyntropin testing was derived statistically with normative data in healthy subjects without acute or chronic illness, and not using clinical criteria with survival outcomes. It is not clear that falling within the reference range established in this fashion truly denotes intact adrenal function with the ability to respond appropriately to the stress of acute illness. Therefore, there is probably a continuum between Groups 2 and 3. Which patients actually have clinically significant adrenal insufficiency is not known. Furthermore, criteria for establishing clinical adrenal insufficiency in hospitalized patients using serum or salivary cortisol responses to cosyntropin are not well established. Therefore, prospective studies with hospitalized patients using different doses of cosyntropin and measuring serum free cortisol, CBG, and salivary cortisol levels are required to demonstrate the true clinical threshold for salivary cortisol that should be deemed "normal" or sufficient.

Salivary cortisol testing does have other potential disadvantages. Intubated critically ill patients can have abnormal salivary flow, and obtaining adequate sample for testing can be difficult [26]. Improperly handled saliva samples can be influenced by topical steroid creams present on the hands of the patients or the hospital staff handling the samples. The salivary glands express 11-beta hydroxysteroid dehydrogenase type 2 (11β-HSD-2) that converts the biologically active cortisol to inactive cortisone. It is possible that altered cytokine levels due to critical illness may have altered 11β-HSD-2 activity which theoretically could have altered the concentration of salivary cortisol we observed [27].

This study has several shortcomings worthy of reiteration. We were forced to use two different doses of cosyntropin (5 and 250 μg) because of a shortage of this drug in the US. This is probably not a major concern since these two doses both provide maximal adrenal stimulation

for both serum and salivary cortisol [12, 20, 28], and there did not appear to be a difference in the response to these two doses within groups of patients. However, a previous study suggested a trend toward a smaller cortisol response to 10 mcg of cortisol compared to 250 mcg in patients with Addison's disease and pituitary tumor, but less so in patients with HPA axis suppression due to glucocorticoid administration [29]. Although all of the subjects were hypoproteinemic, only some had subnormal cortisol responses. As mentioned above, this could be due to subtle differences in adrenal function or possibly differences in CBG concentrations [6], which were not measured in this study. It was not possible to control for the time of day these studies were done, although we are certain that this would not significantly alter the outcome of responses to maximal adrenal stimulation [30]. Finally, this was retrospective study, and a prospective study with more subjects would certainly be needed to confirm our findings. Despite these shortcomings, we feel our study raises a potentially useful approach to the hospitalized patient with suspected adrenal insufficiency. We suggest that the salivary cortisol response to cosyntropin stimulation be performed in hospitalized patients in whom adrenal insufficiency is suspected so that unnecessary, prolonged, and potentially harmful high-dose glucocorticoid therapy can be avoided.

Materials and methods

Subjects

Twenty-eight seriously ill patients with low or low-normal serum albumin and protein concentrations and clinical features or prior and/or concurrent steroid therapy that suggested possible adrenal insufficiency were evaluated by a retrospective analysis of their medical records (Table 1). The reasons for adrenal evaluation by assessment of cosyntropin-stimulated serum cortisol ranged from severe illness with hypotension and infection to milder presentations with hyponatremia and/or a history of prior or concurrent glucocorticoid treatment (Table 1). In many cases (Table 1), prior or concurrent steroid therapy for a variety of illnesses (e.g. multiple myeloma) raised serious concern about suppression of the HPA axis and was the motivation for the cosyntropin-stimulation test. Because of low or low-normal serum albumin (<4.0 g/dl) and protein (<7.0 g/dl) levels, it seemed plausible that an attenuated peak serum total cortisol response to cosyntropin might lead to significant numbers of false positive tests and unnecessary corticosteroid therapy. Therefore, as a surrogate for the measurement of serum free cortisol [13], salivary cortisol was also assessed which is a routine component of our evaluation of possible adrenal insufficiency in critically ill patients with low serum albumin. Thus, the data represent a realistic clinical situation using a typical hospital laboratory. Patient age ranged from 39 to 85 years old. Eleven patients were female, seventeen patients were male. No patients were pregnant or receiving estrogen-containing medication. Sixteen had received prior and/or concurrent steroid therapy including physiological hydrocortisone therapy (stopped >12 h before testing to prevent interference with the cortisol assay), epidural steroid injections within 2 months, dexamethasone therapy (e.g. for multiple myeloma) either for 1–2 weeks before or during testing, Megace therapy, or prednisone (stopped >24 h before testing to prevent interference with the cortisol assay). This prior steroid therapy was suspected as the cause of HPA axis suppression in these patients. Therefore, steroid therapy was discontinued to prevent interference with the serum and salivary cortisol assays rather than to allow HPA axis recovery. None of the patients were intubated at the time of saliva collection.

Study design

We retrospectively reviewed the records of hospitalized patients who had been evaluated with dynamic testing of adrenal function with assessment of serum and salivary cortisol over a 2-year period at a single institution. The decision to subsequently give corticosteroid treatment was determined by the ordering physicians. The use of subsequent glucocorticoid treatment and survival at 1 month following the testing period were used as patient outcomes. A cosyntropin-stimulation test to assess the maximal adrenal response was performed with 250 mcg given to 8 patients and 5 mcg given to the remaining 20 patients. The 5 mcg dose was used because of a shortage of cosyntropin in the United States during the study. The 5 mcg is still a pharmacological dose that gives an equivalent response to 250 mcg; both doses evaluate maximal adrenal function [28]. The time of day at which cosyntropin testing was performed was not controlled for, but was usually between 0600 and 1400.

Analytic methods

Salivary cortisol was collected using a commercially available device (Salivette, Sarstedt, Inc.). Salivary cortisol was measured by enzyme-linked immunoassay (Salimetrics, State College, PA) [15]. The normal salivary cortisol response to cosyntropin was defined as ≥18.0 nmol/l (0.65 mcg/dl) measured 30 min following cosyntropin intravenous injection, which was >2 SD above the mean of the response in healthy subjects [12, 20]. Intra- and inter-assay coefficients of variation of the salivary cortisol assay

were 5.2% and 11%, with a lower limit of detection at 0.3 nmol/l (0.01 mcg/dl). Serum total cortisol was determined using a Bayer Centaur chemiluminescence analyzer. Serum total cortisol was defined with a normal threshold as being greater than 497 nmol/l (18.0 mcg/dl), 30 min following cosyntropin administration which was the cutoff with the best separation between patients with proven adrenal insufficiency and healthy subjects [31].

Statistical analysis

Data are presented as means ± standard error. Data were analyzed by analysis of variance and Duncan's multiple range test with $P < 0.05$ considered significant. Discontinuous data were analyzed by chi-square or one-way analysis of variance on ranks ($P < 0.05$).

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