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Original Article

Intramuscular short-term ACTH test for the determination of adrenal function in children: safe, effective and reliable

Running Title: Effectiveness of intramuscular ACTH in children

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What is already known on this topic?
Intravenos short synacthen test is usually used to detect adrenal insufficiency in childhood

What this study adds?
Intramuscular form of ACTH test can be alternative to intravenos form to detect cases with adrenal insufficiency
Intramuscular form of ACTH test is safe and reliable test with suspected adrenal insufficieny in childhood.

Abstract
Objective: Standart short ACTH stimulation test (SST) has been used for detecting adrenal gland fuction for several years by intravenous (iv). However iv form has not been accessed easily in some countries like ours. The aim of this study was to evaluate the effectiveness of the intramuscular (im) SST.
Methots: Patients were underwent SST with suspected adrenal insufficiency and hyperandrogenism. The SSTs were done with 250 mcg ACTH (Synacthen Depot ampul, concentration 1mg/ml). The cases were divided into two groups: suspected adrenal insufficiency (Group 1 n:87); and hyperandrogenism group (Group 2 n:124). Peak cortisol <18 mcg/dl, was defined as adrenal insufficiency, suspected adrenal insufficiency as a peak cortisol of 18-22 mcg/dl and normal result was defined as a peak cortisol ≥22 mcg/dl.
Results: The mean age of the patients was 11.7±5.2 years. In 164 patients (78%) all of the peak cortisol tests were found normal ≥22 mcg/dl. This rates were 64% and 88% in Group 1 and 2 respectively. Only 8.5% (n=18) of all cases had had an inadequate peak cortisol response of <18 mcg/dl. On follow up, 15 patients needed cortisol therapy whose peak cortisol was <18 mcg/dl. Of all cases 3.3% (n=8) had 17-OHP ≥10 ng/dl. NCCAH clinic findings and/or mutation were found in six of these cases. No local and systemic side effects or allergic reactions were observed in any patient.
Conclusion: Intramuscular form of ACTH test is a safe, effective and reliable test in children with suspected AI. Not seems any local and systemic side effects which supported the reliability of the im ACTH test.
Keyword: Adrenal insufficiency, intramuscular ACTH, childhood, reliability

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Introduction

The clinical presentation of adrenal insufficiency is very variable. Severe overt adrenal failure is usually relatively easy to diagnose however milder adrenal insufficiency (AI) may be much more clinically challenging. Thus dynamic tests are extremely important in detecting subtle (intermediate) AI. It has been recommended to perform dynamic test in every suspected case. Although the insulin tolerance test (ITT) has been used as a gold standard test for the diagnosis of adrenal

insufficiency, it is used with caution because of the risk of seizures in patients with epilepsy and possible morbidity, especially in infants and in patients with cardiovascular diseases (1, 2).
The ITT may be replaced by the SST due to a better safety profile. However the preparations used for a short intravenous (iv) adrenocorticotropic hormone (ACTH) test are not readily available in Turkey and require an official application for prescribing of overseas medicine. Due to this limitation, use of the intramuscular (im) form, which is easily accessible and cheaper, has been used to replace the iv preparation in order to evaluate the adrenal axis of patients without overt adrenal insufficiency.The easily available im-ACTH preparations in Turkey include the original molecule, tetracosactide, known as Synacthen® Depot, which contains 50 units/ml of ACTH (Maalincrodot Speciality Pharmacuiticals Ireland Limited).
The aim of this study was to evaluate the effectiveness of the SST using this preparation and to correlate the findings with the clinical and acquired peak responses and basal cortisol results.

Materials and methods

The data of the patients (such as age, gender, compalints, laboratory results and other demographics) undergoing im-ACTH test between 2010 and 2018 were analyzed from the hospital records system. The inclusion criteria were age <18 years who were tested because of suspected adrenal insufficiency (AI) and all patients who were admitted with hyperandrogenism and underwent SST to investigate the possibility of non-classical congenital adrenal hyperplasia (NCCAH).
The cases were divided into two groups: suspected adrenal insufficiency (Group 1); and hyperandrogenism group (Group 2). Group 1 consisted of cases that had adrenal insufficiency with low basal cortisol and poor cortisol response to insuline tolerance test. Group 2 had presented with findings of virilization such as premature adrenarche, hirsutism and a clinical suspicion of NCCAH (see Figure 1).
The SSTs were done with 250 mcg ACTH (Synacthen Depot ampoule, concentration 1mg/ml) to the patients over two years of age. Blood samples were taken for measurement of cortisol, dehydroepiandrosterone sulfate (DHEAS) and 17-hydroxyprogesterone (17-OHP) at 0, 30 and 60 minutes after administration of the IM-ACTH. Cortisol and DHEAS concentrations were analysed on a Beckman Coulter analyser (Beckman Coulter 250 S. Kraemer Blvd. Brea 92821.USA) by immunoassay method and 17-OHP concentration was measured by using a radioimmunoassay (DIA Source Immunoassay SA Belgium). Peak cortisol <18 mcg/dl, was defined as adrenal insufficiency, suspected adrenal insufficiency as a peak cortisol of 18-22 mcg/dl and normal result was defined as a peak cortisol ≥22 mcg/dl. Genetic analysis for 21 hydroxylase gene mutations were performed in patients with a peak 17 OHP >10 ng/dl. Sensivity, spesifite, PPV (positive predictive value) and NPV (negative predictive value) were calculated. In patients with a definitive diagnosis indicated by the results of the ACTH stimulation test findings on clinical follow-up were investigated.

Statistical Methods

Statistical Package for Social Sciences (SPSS version 22.0 for Windows, Chicago, IL) was used for all statistical analyses. Descriptive statistical analysis of the data was performed. Data distribution was assessed for normality using the "Shapiro-Wilk" test. Data distribution was non-parametric so groups were compared with Mann-Whitney-U test. P <0.05 was considered significant.
The study was approved by the ethics committee of Ankara University with the decision number 02-139-19.

Results

Over the study period 225 patients who had undergone SST were evaluated. Fourteen patients were excluded because of replicated patients and over 18 years old. Thus the final number of study patients was 211. The mean age of the patients was 11.7±5.2 years and 69.2% were female. Of the 211 patients, 87 had been assessed for suspected adrenal insufficiency (Group 1) while 124 patients had been assessed for hyperandrogenism (Group 2). In 164 patients (78%) all of the peak cortisol tests were found normal [≥22 mcg/dl (equivalent to ≥600 nmol/l)]. This proportion was 64% and 88% in Group 1 and 2 respectively. In 29 (13.7%) patients the peak cortisol was 18-22 mcg/dl (equivalent to 500-599.9 nmol/l). Only 8.5% (n=18) of all cases had had an inadequate peak cortisol response of <18 mcg/dl (equivalent to <500 nmol/l).

Group 1 results:

In Group 1, peak cortisol response was <18 mcg/dl in 15 (16%) patients, 18-22 mcg/dl in 19 (21%) patients and ≥22 mcg/dl in 53 (60%) patients (Table 1). Of the patients treated in Group 1, nine had pituitary insufficiency and three had developed AI after cessation of steroid therapy.
Three of the patients with a borderline peak cortisol of 18-22 mcg/dl diagnosed as multiple pituitary hormones deficieny and received treatment. Peak cortisol response was <19 mcg/dl in a further three. In the remaining 16 patients, peak cortisol response was 19-22 mcg/dl and these patients were followed without treatment since there was no clinical finding suggesting cortisol replacement would be appropriate (see Figure 2).

Group 2 results:

In Group 2, peak cortisol response was <18 mcg/dl in three (3%) patients, 18-22 mcg/dl in 10 (8%) and ≥22 mcg/dl in 111 (60%) patients (Table 1). Three of the patients treated in Group 2 with peak cortisol <18 mcg/dl were diagnosed with 17 hydroxylase deficiency and two patients had multiple organ deficiency. One of the patients with a peak cortisol of 18-22 mcg/dl was diagnosed as NCCAH. No patient was detected with a peak cortisol response above 19 mcg/dl who also exhibited clinical signs of AI. On follow up no patient with a peak corisol of > 19 mcg/dl after im-ACTH test required treatment.
A total 24 patients with a peak cortisol <18 mcg/dl after ITT, were evaluated with im-ACTH test and four of 24 (16.7%) patients showed an inadequate cortisol response and treatment was started. Five patients had a peak cortisol response of 18-22 mcg/dl but none of them had any clinical findings and/or risk factors for AI. Fifteen patients had a peak cortisol level ≥22 mcg/dl. When the gold standard is taken as clinical diagnosis; peak cortisol <18 mcg/dl, was defined as adrenal insufficiency; sensivity %93, spesifite %99, PPV: %93 NPV: %99. Peak cortisol <22 mcg/dl, was defined as adrenal insufficiency; sensivity %100, spesifity % 86, PPV: %40, NPV:%100 (see Table 2).
On follow up, 15 patients needed cortisol therapy whose peak cortisol was <18 mcg/dl. Of all cases 3.3% (n=8) had 17-OHP ≥10 ng/dl. NCCAH clinic findings and/or mutation were found in six of these cases. The clinical findings and the test results of the cases were consistent. Mutations were detected in the 21 hydroxylase gene in 6 of 8 (75%) patients who were diagnosed as NCCAH with 17-OHP >10 ng/ml in Group 2 as follows: *V281L* mutation in four, Intron 2 mutation in two, and

the nine most common mutations were not found in the remaining two. No local and systemic side effects or allergic reactions were observed in any patient undergoing im-ACTH test.

Discussion

There is no literature comparing im and iv ACTH tests for the detection of adrenal insufficiency in childhood. In this study, we aimed to assess the effectiveness of the im-ACTH test due to difficulty in obtaining iv-ACTH in Turkey.

Due to limited access to IV Synacthen® (Novartis® Pharma AG 250 mcg/ml) clinicians have to make the diagnosis of AI with basal cortisol and ACTH. Although suspected cases are evaluated with basal ACTH and morning cortisol, this does not always provide a definitive diagnosis. The sensitivity of basal cortisol for the diagnosis of AI is only 60% (2). There are a number of reasons for this. These include immaturity of the hypothalamo-pituitary-adrenal (HPA) axis, very young patients, innaccurate sampling times of the morning cortisol which, because of diurinal cortisol variation, should be performed at a consistent time and thus differences between studies can be difficult to reconcile.

There are some adult studies describing assessment of the HPA axis with im-ACTH due to difficulties in obtainig the IV form and the poor reliability of the diagnosis of AI based on basal cortisol values. The efficacy of im-ACTH has been evaluated in detecting both primary and central AI cases in India (3). Gundgurthi *et al*, performed STT tests with im-ACTH (25 U im preformed with Acton). Two groups were identified; the validation group which included healthy volunteers, diabetes mellitus, hypothyroidism and adrenal insufficiency and the study group which included all patients who were tested with the diagnosis of AI. Although the basal cortisol level was less than 3 mcg/dl, it was diagnostic, but in the study, 40% of the patients diagnosed with AI and only 60% of these patients had basal cortisol levels below 3 mcg /dl. In seven cases with a baseline cortisol value <3 mcg/dl, the delta, defined as the difference between basal and peak cortisol in the test, increased by 7 mcg/dl on stimulation. In eight cases, there was less increase but basal cortisol level was found to be >3 mcg/dl. Subnormal increases have a similar sensivity to basal cortisol and have lower specificity and positive predictive value. The authors concluded that the diagnosis of AI with im-ACTH form was superior to basal cortisol values and the HPA axis could be assessed with these tests in many health institutions. In addition im-ACTH test may be preferred because it was cheap and easily accessible.

Among our cases, 11 cases with basal cortisol ≤3 mcg/dl indicating suspicion of adrenal insufficiency were found. In only six of these eleven cases, peak cortisol value was <18 mcg/dl and only seven cases were followed up with treatment.

Although there are limited studies in children, there are studies to detect effectiveness IM and IV forms in adults. Keleştimur *et al*. evaluated im and iv forms of ACTH in their study (4). Twenty healthy adults were tested with IV Synacthen® and two weeks later with Depot Synacthene R. Thus the authors evaluated peak cortisol acquisition times and cortisol increase rates in the same volunteers using both iv and im forms of ACTH. Samples were taken at 0, 30, 60, 90 and 120 minutes and the time to obtain cortisol increase and peak response were similar. They concluded that depot forms of ACTH for SST can successfully replace iv Synacthen®. In 90% of cases tested with iv Synacthen®, peak responses were obtained 60 min and after and with the IM form peak responses were detected at 90 minutes and before. When the peak response was taken as 22 mcg/dl, the peak response was obtained in 95% of the patients who received both the IV and IM form at 90 minutes or before. The only difference was that peak responses were concentrated at 30 minutes with the iv form and at 60 minutes using the IM form. Regardless of the form of synacthen® used, there is no need to prolong the test to 120 minutes or more. In our study, blood was taken at 0, 30 and 60 minutes with the IM form and peak responses were reached at 60 minutes. In our study, sampling was stopped after the 60 minute sample.

Data obtained from adult studies are similar (5). Women of reproductive age (n=29) were given ACTH either iv or im, cortisol and androgen precursors were tested and no difference was found between the stimulated peaks of the two forms (4). In another study, using IM and IV forms, cortisol increment was compared with serum levels in every 15 minutes, peak cortisol levels were obtained for both iv and im-ACTH forms at 60 minutes and the two forms were reported to be no different (5).

There are also studies attempting to determine the lowest effective dose of IM-ACTH for the stimulation of the HPA axis. In one study, 21 healthy volunteers, nine primary adrenal AI and ten secondary AI were given im-ACTH (250 mcg/ml Synacthen® NovartisR Pharma AG) at a standard dose of 250 mcg im into the deltoid muscle and blood, saliva cortisol and aldosterone concentrations were measured (6). In this study the doses were varied and titrated so that 12.5, 25 and 250 mcg doses were compared. In healthy humans, 30 minute cortisol response was the same after 25 and 250 mcg im while 12.5 mcg was not sufficient to detect AI. Neither did the 30 minute salivary cortisol concentration differ between the 25 and 250 mcg im injections. However, there was no correlation between salivary and blood cortisol levels. The responses were same no change in serum and salivary cortisol levels after low (25 mcg) and high dose (250 mcg) im ACTH in patients with adrenal insufficiency.

The effectiveness of the im-ACTH test in detecting central AI cases was also investigated (8). Cases with short stature and at risk of having multiple hormone deficiencies and tested with ITT were evaluated. Twenty cases were identified because of ITT peak cortisol below <18 mcg/dl. All cases who responded poorly to ITT were re-evaluated one week later with 25 U ACTH and cortisol responses in both tests were compared. Although the peak responses were similar, an adequate response was obtained by im ACTH in six cases although there was insufficient response by ITT. This was attributed to the injection of ACTH at supraphysiological doses. A further finding of this study was that when the cut-off value was taken as 18 mcg/dl, sensivity was 57% with a specificity of 94% whereas the sensitivity increases to 100% when the cut-off was 22 mcg/dl although the specificity fell to 75%. Thus the diagnosis of AI with a peak cortisol <18 mcg/dl by im ACTH was robust whereas high reliability was ruled out at peak values above 22 mcg/dl. In our cohort, in 24 cases with ITT cortisol response <18 mcg/dl, when the im ACTH test was done subsequently and the peak response was between 18-22 mcg/dl in five patients and above 22 mcg/dl in 15 of them. Only four patients underwent hydrocortisone replacement. There are two possible explanations. Firstly, the dose used for im ACTH may be a supraphysiological dose for central adrenal insufficiency detection. Secondly, there was no central adrenal insufficency in any of the patients with a peak cortisol level <18 mcg/dl by ITT. As the ITT response is age-variable and threshold values are not be standard for all ages. This is supported by the finding that higher peak values were obtained in healthy children under the age of 12 years (9). Our second hypothesis

supports the lack of AI findings in any of our patients who had insufficient response with ITT and had adequate response with im ACTH test.
The most important limitation of our study was that retest with iv ACTH could not be performed to evaluate the correlation with the im form of ACTH in our patients.
In conclusion we have shown that the im form of ACTH test in children with suspected AI is a safe, effective and reliable test. None of the patients with peak cortisol levels ≥22 mcg/dl were found to have AI on long-term follow-up. In contrast, peak responses <18 mcg/dl were diagnosed as AI whereas responses above 22 mcg/dl excluded the diagnosis of AI.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of Ankara University with decision number 02-139-19
Informed Consent: -

Authorship Contributions

Surgical and Medical Practices: Prof. Dr. Merih Berberoğlu, Prof.Dr. Zeynep Şıklar
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Data Collection or Processing: Dr. Elif Özsu, Dr.Esra Bilici, Dr. Aysegul Ceran, Dr. Rukiye Uyanık
Analysis or Interpretation: Prof.Dr Zeynep Şıklar, Prof. Dr. Zehra Aycan, Dr. Elif Özsu
Literature Search: Uzm. Dr. Elif Özsu, Uzm. Dr.Esra Bilici, Uzm.Dr. Aysegul Ceran,.Dr. Rukiye Uyanık, Dr. Tuğba Çetin
Writing: Prof. Dr Merih Berberoğlu, Prof.Dr Zeynep Şıklar, Dr. Elif Özsu

Conflict of Interest: No conflict of interest

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Table 1: Demographic and biochemical characteristics of patients with IM-ACTH test
(*: p<0.05)

	Group 1 (Suspected Adrenal deficieny) (n 87)	**Group 2 (Hiperandrogenism) (n 124)**	**p**
Age (decimal age)*	10± 5,4 (0,14-18,9)	12,9± 4,7(0,32-23,8)	<0.05
Sex (female /male)	44/43	102/22	
Basal cortisol (mcg/dl)*	8,6± 4,2 (0,1-19)	15,9± 7,4 (3,9-52)	<0.05
Stimulated cortisol (mcg/dl)*	15,9± 7,4 (3,9-52)	29,3 ±7,2(3-53)	<0.05
Peak cortisol <18 mcg/dl (n 18) 18-22mcg/dl (n 29) ≥22 mcg/dl (n 164)	 %16 (N: 15) %21 (N: 19) %60 (N: 53)	 %3 (N: 3) %8 (N:10) %89 (N: 111)	
Basal 17OHP(ng/dl)*	0,86± 0,9 (0,1-5)	0,86± 0,9 (0,1-5)	<0.05
Stimulated 17OHP (ng/dl)*	2,7±1,7 (0,42-9,44)	5,1 ±6,7 (1-50)	
Beginning treatment	%18 (16)	%6 (7)	
Not on treatment	%84 (71)	%94 (117)	

Table 2: Threshold value after im-ACTH in patients with ITT and peak cortisol <18 mcg/dl

	Adrenal Insufficieny	**No Adrenal Insufficieny**
IM ACTH <18mcg/dl <22 mcg/dl	 N: 14 N: 17	 N: 1 N: 25
≥18 mcg/dl ≥ 22 mcg/dl	N: 1 N: 0	N: 163 N: 163

Peak cortisol <18 mcg/dl, was defined as adrenal insufficiency; *sensivity %93, spesifite %99 PPV: %93 NPV: %99.*
Peak cortisol <22 mcg/dl, was defined as adrenal insufficiency*; sensivity %100, spesifity % 86, PPV:%40, NPV:%100*

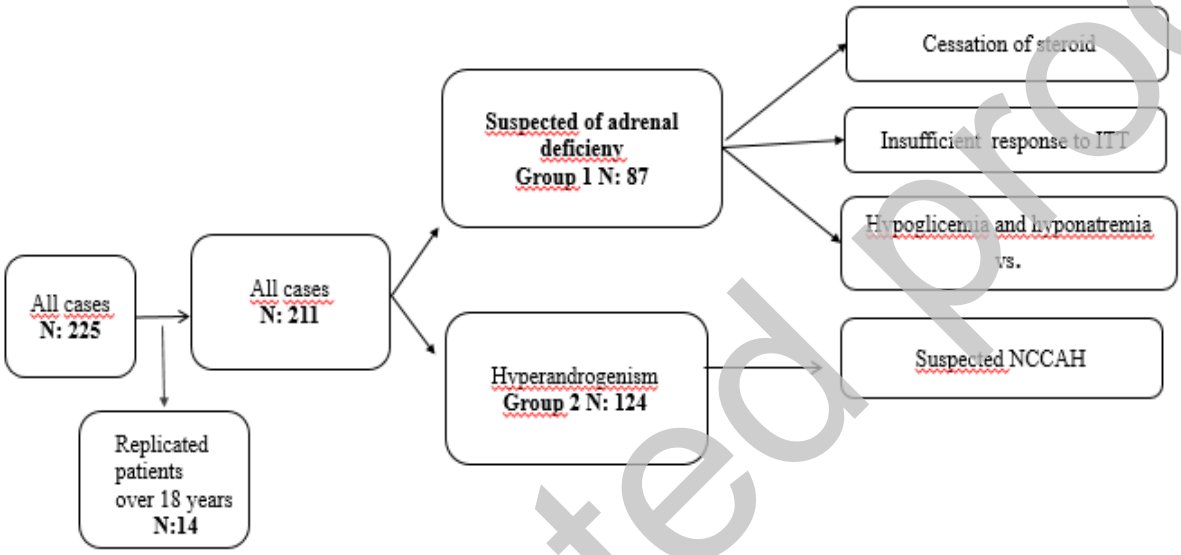

Figure 1: Classification of selected cases according to etiological factors

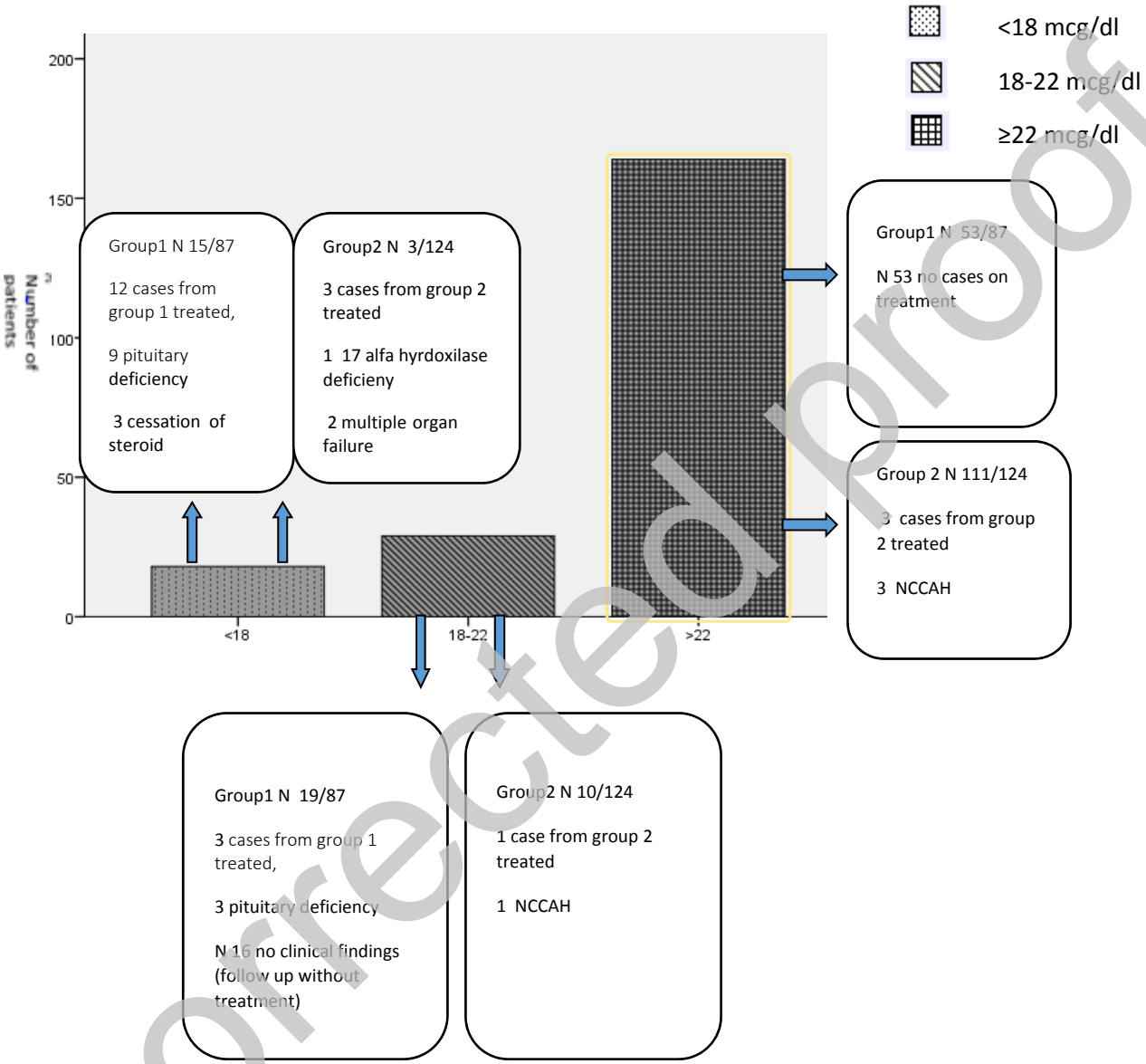

Figure 2: Number and treatment status of all cases according to peak cortisol level