

## Characteristics of Glucocorticoid Resistance in Ankylosing Spondylitis

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| Article History                                                                                                         | Abstract                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |
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| <p>Received: 06 June 2023<br/>Revised: 05 Sept 2023<br/>Accepted: 06 Dec 2023</p> <p>CC License<br/>CC-BY-NC-SA 4.0</p> | <p><i>Despite the fact that significant progress has been made in the treatment of ankylosing spondylitis, and basic drugs (methotrexate, sulfasalazine, etc.) are widely used, as well as various genetically engineered drugs that make it possible to achieve remission of the disease, glucocorticoid hormones continue to be one of the means of treating the disease, because they allow to achieve a sufficiently rapid decrease in the activity of the disease, as well as in the presence of systemic manifestations. Their use allows to reduce inflammatory activity, to stop the symptoms of inflammation and thereby to ease the condition of patients. The authors have established a different degree of sensitivity to various glucocorticoids, which must be taken into account during treatment.</i></p> <p><b>Keywords:</b> Ankylosing Spondylitis, Glucocorticoids, Lymphocytes</p> |

### 1. Introduction

Annually in the world more than 150 million people take glucocorticoids due to various diseases that require their constant intake (Nasonov E.L., Folomeeva O.N., Silverman N.M., 2014). Despite the fact that glucocorticoids are not the main means in the treatment of ankylosing spondylitis, they are very widely used in this disease. Their use is justified if patients have systemic manifestations (uveitis, carditis, vasculitis), recurrent synovitis, a high degree of disease activity accompanied by fever. The use of glucocorticoids makes it possible to stop the main manifestations of the inflammatory reaction in a short time and improve the course of the disease, but does not allow to stop the progression, and therefore their widespread use is inappropriate. For almost 70 years of the history of the use of glucocorticoids in rheumatology, including in the treatment of ankylosing spondylitis. There are enough facts accumulated that indicate the presence of non-sensitivity (resistivity) of the patient to glucocorticoids in patients [1]. However, until recently, this condition was detected empirically and there were no methods to assess sensitivity to glucocorticoids, which complicated the situation.

Over the past 70 years since the beginning of the use of glucocorticoids in the clinic of internal diseases, a huge experience of their use has been accumulated, but at the same time, quite a lot of facts have been noted indicating that patients have resistance to certain glucocorticoids. Moreover, it is noted as primary resistance, which is detected immediately when taking glucocorticoids, and secondary – occurring against the background of a certain period of glucocorticoid use. Often these signs turn out to be erased, which makes it quite difficult to diagnose this condition and, as a consequence, to treat the disease, the possibility of replacing it with another glucocorticoid drug. Naturally, complications of therapy develop, sometimes exceeding the severity of the disease, sometimes leading to a tragic ending. Naturally, this causes unjustified fear of the appointment of glucocorticoid therapy and unjustified refusal to prescribe this category of drugs, which in turn leads to a worsening of the course of the disease, in which hormones are the drugs of choice. To date, the appointment of hormones is carried out empirically, based on the experience of a doctor, and information gleaned from textbooks and manuals and the effectiveness of treatment is determined only *ex juvantibus*. The above situation is caused, firstly, by the lack of detailed data on the mechanisms of hormone resistance development. Purely empirically, it is noted that there is primary hormone resistance, and sometimes secondary develops (Shimanovsky N.L., 2010). However, data on the frequency of development of such a condition remain completely unknown. In addition, it remains unknown how sensitivity to glucocorticoids changes, depending on the nosology, duration of illness,

degree of activity, as well as the presence of concomitant illness. The role of genetic factors also remains unknown, and which gene or group of genes is responsible for the development of such a condition. Secondly, the lack of reliable methods for determining sensitivity to glucocorticoids in patients. To date, almost only six glucocorticoids have been created that are used for systemic use. These are hydrocortisone, prednisone, methylprednisolone, dexamethasone, triamcinolone and betamethasone. It is generally believed that steroid resistance is understood as the preservation of signs of activity for 4 weeks during treatment with prednisone at a dose of 0.75 mg / kg / day. To date, there have been no acceptable methods for determining sensitivity to glucocorticoids, and the created ones were extremely inconvenient in execution, required a large amount of equipment and reagents, and at the same time the determination was performed to one glucocorticoid, most often to dexamethasone. To date, there have been no methods for simultaneous determination of sensitivity to glucocorticoids in peripheral blood. And the appointment of drugs was carried out empirically, based on his personal experience as a doctor and ideas about the treatment of a particular disease, including information gleaned from various textbooks and manuals on internal medicine, which in turn could not improve the situation with the appointment of hormones. Glucocorticoids have various effects on blood cells, and the phenomenon of cortisol-resistance of lymphocytes, i.e. such lymphocytes that do not die under the action of cortisol, has long been known. That is why the use of lymphocytes as markers of sensitivity to glucocorticoids seems promising [2,3,4]. Previously, various researchers attempted to develop methods for determining sensitivity to glucocorticoids, but these methods turned out to be time-consuming, required a large number of reagents and complex devices, and were limited mainly to determining sensitivity to any one hormone, mainly dexamethasone. In this connection, they have not found wide application in clinical medicine. The aim of study was to study the nature of sensitivity to glucocorticoids in patients with ankylosing spondylitis.

## 2. Materials And Methods

15 patients with ankylosing spondylitis were examined, 2 of them women. The average age was  $43.29 \pm 2.37$  years, the duration of the disease was  $15.45 \pm 2.41$  years, who had the II degree of disease activity (ESR- $29.35 \pm 1.98$  mm/h, C-reactive protein  $-26.6 \pm 2.01$  mg/l). The diagnosis of ankylosing spondylitis was established according to the New York criteria (1984). All patients had stage II-IV sacroileitis. Previously, patients took various glucocorticoids: prednisone, dexamethasone, methylprednisolone and another were hormone-dependent and took, in terms of prednisone, at a dose of  $12.5 \pm 1.5$  mg / day. Patients also took various anti-inflammatory drugs, immunosuppressants, biological genetically engineered drugs (Remicade, Actemra, Secunimab). All patients underwent blood testing for sensitivity to glucocorticoid hormones for 2-3 days of stay in the clinic. Sensitivity to hydrocortisone, prednisolone, methylprednisolone, dexamethasone, triamcinolone, betamethasone (i.e., to almost all known glucocorticoids used in rheumatology) was determined. The determination of sensitivity to glucocorticoids was carried out as follows: 4 ml of venous blood into a sterile heparinized centrifuge tube, centrifuged for 10 minutes at 1500 rpm, lymphocytes were isolated by the Boum method at 76% ficollet and the number of lymphocytes in the Goryaev's chamber was counted under a microscope, then added to individual test tubes using measures 500 ml of lymphocyte suspension are pipetted and 100 ml of betamethasone, dexamethasone, prednisone, hydrocortisone, triamcinolone and methylprednisolone are added to each tube using separate measuring pipettes,. The resulting mixture is incubated in a thermostat at a temperature of  $37^{\circ}\text{C}$  for 1 hour, then stained with trypan blue and fixed, after which the remaining lymphocytes are counted in the Goryaev's chamber under a microscope, then sensitivity to glucocorticoids is determined depending on the number of remaining lymphocytes, while if the number of the number of lymphocytes that are not bound to glucocorticoid is more than 90%, then the sensitivity to glucocorticoid is estimated as very low if the number of lymphocytes that are not bound to glucocorticoid is 70-90%, then the sensitivity is assessed as low, if the number of lymphocytes not bound to glucocorticoid is 50-70%, then the sensitivity to glucocorticoid is assessed as average, if the number of lymphocytes not bound to glucocorticoid is 25-50%, then the sensitivity to glucocorticoid is assessed as high, if the number of lymphocytes not bound to glucocorticoid is, if there are patients with glucocorticoid, it is less than 25%, then the sensitivity to glucocorticoid is estimated as very high. This technique has been developed and patented by the authors in the Republic of Uzbekistan. Ampulated solutions of glucocorticoids were used. Statistical processing was performed using the Exel software package.

## 3. Results and Discussion

The data obtained are shown in the table.

**Table 1:** The nature of the distribution of sensitivity to glucocorticoids in patients with ankylosing spondylitis

| <b>Drug<br/>the character is<br/>sensitive<br/>the yields</b> | <b>betameta<br/>zone</b> | <b>methyl<br/>prednisolone</b> | <b>dexa<br/>metha<br/>zone</b> | <b>predn<br/>izolone</b> | <b>triamci<br/>nolone</b> | <b>hydrocor<br/>thizon</b> |
|---------------------------------------------------------------|--------------------------|--------------------------------|--------------------------------|--------------------------|---------------------------|----------------------------|
| Very high                                                     | 3                        | 1                              | 6                              | 3                        | 3                         | 1                          |
| High                                                          | 4                        | 3                              | 4                              | 4                        | 3                         | 2                          |
| Medium                                                        | 5                        | 4                              | 1                              | 3                        | 3                         | 3                          |
| Low                                                           | 2                        | 5                              | 2                              | 3                        | 3                         | 4                          |
| Very low                                                      | 1                        | 2                              | 2                              | 2                        | 3                         | 5                          |

From the data in the table it can be seen that the largest number of very highly sensitive patients are dexamethasone, followed by betamethasone, prednisone and triamcinolone, and at the end are hydrocortisone and methylprednisolone. Among the highly sensitive, the first place was distributed between betamethasone, dexamethasone and prednisone, methylprednisolone and triamcinolone are in second place, and hydrocortisone is at the end. Betamethasone takes the first place among the medium-sensitive ones, followed by methylprednisolone, then prednisolone, triamcinolone and hydrocortisone take the place equally, and hydrocortisone is located at the end. Among the low sensitive ones, methylprednisolone is in the first place, hydrocortisone is in the second place, then prednisone and triamcinolone are next, and betamethasone and dexamethasone are in the end. Among very low-sensitivity patients, hydrocortisone came first, followed by triamcinolone, followed by methylprednisolone, dexamethasone and prednisone, and betamethasone was placed at the end. The authors obtained the first data on the nature of glucocorticoid resistance in ankylosing spondylitis, which may change with the accumulation of data. It should be noted that the same patient has different sensitivity to various glucocorticoid hormones.

One of the main cells of the immune system that are affected by glucocorticoids are lymphocytes located in peripheral blood and tissues. The presence of glucocorticoid receptors in lymphocytes is well known – the main immunocompetent cells involved in both the immune response and the perception of glucocorticoids – both endogenous, produced by the adrenal glands, and exogenous, obtained from the outside. (Morris S. C. et al., 2006). The phenomenon of cortisol-resistant lymphocytes in bronchial asthma has long been known, i.e. such lymphocytes that do not die and do not change when exposed to endogenous or exogenous cortisol. To date, there is no data on the nature of glucocorticoid sensitivity in patients with ankylosing spondylitis. The mechanisms of the development of hormone resistance to glucocorticoids are different. Firstly, these are defects in the shapeyron-protein located on the surface of the lymphocyte, with the help of which the glucocorticoid penetrates into the cell [5,6]. And, secondly, genetic factors leading to mutations of genes responsible for the development of glucocorticoid resistance [1,7,8]. The authors have not found in the available literature information about the nature of glucocorticoid resistance to various glucocorticoids simultaneously, not only in ankylosing spondylitis, but also in other diseases requiring glucocorticoid therapy [9,10].

The true frequency of hormone resistance in ankylosing spondylitis is still unknown, but the results show that these patients have different degrees of sensitivity to hormones According to various authors, the frequency of hormone resistance can reach 30% [2,11,13], which in absolute numbers represents a large number of patient. Currently, the problem of hormone resistance is highlighted in bronchial asthma, rheumatoid arthritis, systemic lupus erythematosus. According to N.L. Shaporova, the frequency of resistance to GCS can reach 30-40% in AD, and 30% in rheumatoid arthritis [11]. It should be noted that Hashimoto (2012) [12] provides retrospective data on the nature of glucocorticoid sensitivity in 1125 patients with system lupus erythrimatosis since 1955. At the same time, the author characterized a different degree of individual sensitivity to steroids, mainly to hydrocortisone, prednisone and dexamethasone. Moreover, the degree of sensitivity/resistance in patients was carried out empirically and in the process of monitoring the indicators of disease activity. Currently, the mechanisms of the development of glucocorticoid resistance are insufficiently studied. In 1985, the gene responsible for the development of glucocorticoid resistance and its polymorphism was established. BP DNA was cloned and two genes encoding the alpha and beta isoforms of BP were identified, and recently a third isoform of BP responsible for the development of resistance to glucocorticoids, which in some tissues is expressed even more strongly than the alpha and beta isoforms, was discovered. When binding glucocorticoids, the alpha isoform forms homodimers and in

this form interacts with hormone-sensitive DNA elements. It turned out that hormone-receptor complexes regulate gene expression by binding to DNA not only in the form of dimers, but also heterodimers. The latter can cause effects other than those of dimers (Orbak E, 2006). It has also been established that corticoid-dependent globulin (CBG), a poly-drug resistant transporter (MDR) and the enzyme 18 $\beta$ -hydroxysteroid dehydrogenase participate in the development of glucocorticoid resistance (Borowski L.C., 2007; Silverman M.N., 2008; Straub H., Dukhanin E.L., 2014). According to Straub R.H (2014), there is a certain relationship between the level of GC intake and the development of glucocorticoid resistance. But the study of the mechanisms of glucocorticoid resistance is far from final resolution. And with systemic lupus erythematosus in the implementation P-glycoprotein and the multi-drug resistant substance MDR1 are involved, which also participates in the formation of resistance in rheumatoid arthritis (Silverman M.N., 2011. Goes S., 2014).[13]

Previously, methods for assessing sensitivity to glucocorticoids were based on the assessment of glucocorticoid sensitivity receptors, the level of pro-inflammatory cytokines, various genes and their polymorphism, and most often to one glucocorticoid and in one disease. The methods were complex, time-consuming and required sophisticated equipment and reagents. The main disadvantage of all of the above methods was the determination of sensitivity to glucocorticoid alone (most often dexamethasone), the need for various reagents and complex equipment, and as a result, the complexity of execution and limited scope of application. That is why the development of a technique for simultaneous determination of sensitivity to several glucocorticoids in various autoimmune rheumatic diseases is an actually task. The results obtained showed an unequal degree of sensitivity to various glucocorticoids, which must be taken into account when prescribing them. Taking into account the nature of sensitivity, glucocorticoids were replaced in 5 patients, taking into account sensitivity, which quickly led to an improvement in the clinical condition and normalization of laboratory parameters.

#### 4. Conclusion

Patients with ankylosing spondylitis have varying degrees of sensitivity to glucocorticoids. The same patients has a different degree of sensitivity to various glucocorticoids.

#### Conflict of interest

The authors declare that there is no conflict of interest

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