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INTRODUCTION

Subglottic stenosis (SGS) is a rare and recalcitrant fibroinflammatory disease resulting in cicatrical narrowing of the upper airway. Although classically treated surgically, resection and dilatation can be problematic given the chronic nature of the disease. Endoscopic resection and dilatation procedures often require multiple procedures per year and contribute to the morbidity experienced by patients.1,2 Although large series have demonstrated the sustained benefit of cricotracheal resection in select patient groups,3,4 these procedures are more morbid, with greater risk of pneumothorax and detriment to vocal pitch and projection.5,6 Additionally, many patients are unable to undergo these extensive procedures, and some will experience restenosis afterward, especially in idiopathic and autoimmune cases, where the underlying disease process has not been addressed.7,8

Clinical and histological examination of stenotic segments indicates that SGS is driven by a chronic inflammatory process.9–11 Inflammation seemingly promotes an imbalance in extracellular matrix synthesis and degradation, as witnessed in other cicatrical conditions such as keloid or hypertrophic scarring.5,12 A greater understanding of the drivers of this disease has led to increasing interest in scar-modification therapy.

Multiple medical approaches to scar reduction and modification in SGS patients have been proposed in both animal and human models. These include the use of mitomycin C,13 rapamycin,14 5-fluouracil,15,16 and steroids.2,7 Franco et al. recently demonstrated the effectiveness of serial intralesional steroid injection (SILSI) and its ability to improve airway caliber independent of other therapeutic interventions in idiopathic SGS.11 Glucocorticoids are known to quell inflammation and fibroblast proliferation, while simultaneously encouraging the degradation of collagen, and in this way target the fundamental components of poor wound healing demonstrated in SGS.17,18

Steroids, while being potent anti-inflammatory, have many systemic side effects. As such, an additional potential benefit of intralesional steroid injection is the theoretically limited systemic bioavailability and systemic, steroid-related adverse events. Given the serial nature of SILSI, it is crucial to demonstrate that any systemic effects, particularly hypothalamic–pituitary–adrenal (HPA) axis

**Objectives/Hypothesis:** Serial intralesional steroid injection (SILSI) has recently been proposed as an effective scar-modifying therapy for subglottic stenosis (SGS). We aimed to explore the systemic absorption of steroid following SILSI and to characterize the magnitude and chronicity of any effect observed. Specifically, we aimed to show that any effect resolves prior to the next intralesional injection.

**Study Design:** Prospective, observational pilot study.

**Methods:** Patients were injected intralesionally with 40 to 200 mg triamcinolone. Serum cortisol, as well as white cell counts and inflammatory markers were measured at day 0 (baseline), 1, 7, and 28. Salivary cortisol was measured at baseline and for 7 consecutive days following injection.

**Results:** Six patients with idiopathic SGS were recruited. At baseline, serum cortisol measured 284.0 ± 61.4 nmol/L and fell significantly to 15.5 ± 4.3 nmol/L 1 day following triamcinolone injection (P = .03). At day 7, serum steroid levels showed significant recovery to 221.8 ± 78.9 nmol/L (P = .03) and further rose to 279.5 ± 29.9 nmol/L at 28 days (P = .07). Salivary cortisol exhibited a similar pattern with significant recovery by day 6 (P = .04) and suggestion of exponential clearance of triamcinolone systemically. White cell counts were also affected by systemic absorption of exogenous steroid. No significant change in inflammatory markers was observed.

**Conclusions:** Our findings demonstrate systemic absorption of steroid following SILSI, with acute hypothalamic–pituitary–adrenal (HPA) axis suppression. However, normalization of HPA axis function by day 7 suggests that although acute steroid side effects should be discussed with patients, no cumulative systemic steroid side effect would occur with serial injections.

**Key Words:** Subglottic stenosis, airway, intralesional, steroid, serial intralesional steroid injection, safety, hypothalamic–pituitary–adrenal axis.

**Level of Evidence:** 2
suppression, resolve prior to the administration of the next intralesional injection, so as to avoid cumulative effects. To this end we have, for the first time, examined the extent of steroid absorption following SILSI and its effect on adrenal, immune, and inflammatory markers of systemic activity over time.

**MATERIALS AND METHODS**

A prospective, single-arm, observational pilot study was designed to assess the effect of SILSI on systemic markers of steroid absorption following a single injection. Adult SGS patients undergoing SILSI therapy were recruited. Patients who were pregnant, suffered from conditions with direct effects on HPA axis function, or who were exposed to glucocorticoids in the previous 30 days were excluded from the study. Testing occurred parallel to the SILSI procedure, which involved awake, in-office injection of 1.0 to 5.0 mL of triamcinolone acetonide 40 mg/mL (40–200 mg), subepithelially, into the stenotic segment every 4 weeks, as described by Franco et al.1

Blood collections occurred at baseline (day 0) on the morning of the procedure, as well as 1 and 7 days following the procedure. If day 7 serum cortisol was found to be lower than the day 1 value, a further full round of testing occurred on day 28 (Fig. 1). All blood tests occurred between 0800 and 0830 hours. Patients were also asked to collect 1.0 to 2.0 mL of saliva each morning, from day 0 to day 7, for salivary cortisol testing.

Key time points of 0, 1, and 7 days were chosen based on previous intralesional steroid-safety studies, investigating intrarticular19,20 and intradermal21,22 administration. Based on these studies, we hypothesized that peak effect would be seen at day 1, and resolution would occur within the first week. These values were compared to baseline results derived from day 0 testing in each patient. Day 28 testing functioned to ensure that any potential HPA axis suppression observed at day 7 resolved prior to the next injection.

Hematological investigations included serum cortisol, white cell counts, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Salivary free cortisol was assayed from daily saliva samples.

Basal tests of HPA axis function, such as serum, and salivary cortisol were used as particularly sensitive markers of steroid absorption. Salivary cortisol allowed for convenient, frequent sampling and closer, daily assessment of the effect of exogenous steroid administration on endogenous cortisol production in the first week after treatment.

The effect of glucocorticoids on immune function is well documented, and white cell count, in particular eosinophil and lymphocyte counts, have been identified as relatively sensitive markers of steroid absorption.23–25 CRP and ESR were measured as surrogate markers of systemic inflammatory activity.

Serum cortisol was analyzed using the Beckman Coulter DxI 800 (Beckman Coulter, Inc., Brea, CA) instrument, whereas CRP and white cell counts were analyzed using the Beckman Coulter AU5812 (Beckman Coulter, Inc.) instrument. ESR was measured by the modified Westergren method. Salivary cortisol was measured using high-sensitivity liquid chromatography–tandem mass spectrometry.

Test results across time points have been reported for each patient. Descriptive statistics were used to present mean ± standard deviation. A Wilcoxon signed rank test was used to assess the significance of change from baseline over time in salivary and serum cortisol. $P$ values have been ascertained for changes between day 0 and 1, day 1 and 7, as well as day 1 and 28. The project was carried out in accordance with the Declaration of Helsinki and was approved by the Monash Health Human Research Ethics Committee (LNR/17/MonH/102).

**RESULTS**

Six patients met all inclusion and no exclusion criteria and were recruited to the study.

All patients were female and had idiopathic subglottic stenosis. The mean age was 49.7 ± 10.1 years, and the mean body mass index was 33.0 ± 6.5 kg/m². Results of tests performed on blood have been summarized in Table I. Salivary cortisol results have been summarized in Table II.

**HPA Axis Function**

Serum cortisol (Table I) had a baseline value of 284.0 ± 61.4 nmol/L at day 0. On day 1 post-injection, mean serum cortisol had fallen significantly to 15.5 ± 4.3 nmol/L ($P = .03$). By day 7, significant recovery of HPA axis function occurred (221.8 ± 78.9 nmol/L, $P = .03$). Although less than baseline, this result is nonetheless in the normal range for the morning cortisol assay used (150–750 nmol/L). By day 28, the mean serum cortisol level returned to baseline at 279.5 ± 29.9 nmol/L ($P = .07$). Inspecting the individual patient traces, patients 2, 3, and 4 all showed complete recovery of HPA axis suppression by day 7. Meanwhile, patients 1, 5, and 6 did not, the latter two showing marked suppression 1 week following SILSI. Further recovery to normal levels occurred by day 28 (Fig. 2).

Daily salivary cortisol levels at baseline were 8.7 ± 4.0 nmol/L (Table II), similarly showing significant suppression at day 1 (0.3 ± 0.3 nmol/L, $P = .04$), but suggesting substantial recovery by day 5 (4.0 ± 2.7 nmol/L, $P = .08$) and full, significant recovery by day 6 (8.8 ± 7.0 nmol/L, $P = .04$). Overall patients demonstrated a return to baseline by day 5 to 6, with one patient achieving a return to baseline as early as day 2 (Fig. 3).

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**Fig. 1.** Testing timeline. SILSI = serial intralesional steroid injection.

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**Table I.** Salivary cortisol results have been summarized in Table II.
Immune Function

Total white cell, neutrophil, lymphocyte, and eosinophil counts were measured. A similar trend of deranged cell counts at day 1 with resolution back to baseline by day 7 was observed. Circulating neutrophils increased from 3.1 ± 0.5 at baseline, to 8.1 ± 2.3 × 10⁹/L at day 1 under the influence of exogenous steroids. Recovery to 4.1 ± 1.5 × 10⁹/L was observed at day 7. Conversely, lymphocyte count was reduced one day following SILSI from 1.7 ± 0.8 to 1.2 ± 0.5, and showed recovery to 2.0 ± 1.1 × 10⁹/L at day 7. Circulating eosinophils behaved similarly, with a reduction from 0.14 ± 0.07 to 0.01 ± 0.02 and full recovery to 0.13 ± 0.10 × 10⁹/L (Table I) (Fig. 4).

Inflammatory Markers

SILSI had no discernable effect on CRP or ESR levels, as demonstrated by the relatively unchanged mean values and high degree of variability in individual values (Table I).

DISCUSSION

Intralesional steroid injection, as an adjunct to surgery, has been a part of the SGS treatment paradigm since the early 1970s. However, only recently, serial intralesional steroid injection without surgery has, in certain cases, been shown to significantly slow the progression of SGS, and in some instances actually improve the subglottis with the intention of characterizing the duration and magnitude of any systemic effect.

Though widely used for intralesional injection, we had some preliminary concerns about the use of triamcinolone serially. Triamcinolone acetonide is a lipophilic formulation and is biologically inactive until it is deacetonized and rendered hydrophilic. It is therefore theorized to remain in tissue and act locally for longer periods than primarily hydrophilic preparations such as dexamethasone phosphate. This subsequently prolongs the tissue half-life and its duration of action in the subglottis. With this in mind, the question naturally arises whether a lipophilic steroid salt might also confer longer-lasting systemic effects and thus be unsuitable for serial injection. However, the results of our prospective study demonstrate that noticeable changes in baseline levels of systemic biomarkers resolve as early as day 2 in one case and in general by days 5 to 7 post-injection.

During this time, significant changes in serum and salivary cortisol levels were observed; however, recovery occurred within 1 week of administration. Through measurement of daily salivary cortisol, we have shown that patients largely recover cortisol homeostasis by day 6. Three patients, 1, 5, and 6, showed residual suppression of HPA axis function on day 7 serum cortisol.

Salivary Cortisol Values Over Time.

<table>
<thead>
<tr>
<th>Day</th>
<th>Day 0, n = 5</th>
<th>Day 1, n = 6</th>
<th>Day 2, n = 7</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary cortisol, nmol/L</td>
<td>8.7 ± 4.0</td>
<td>0.3 ± 0.3*</td>
<td>1.4 ± 2.2</td>
<td>2.0 ± 3.1</td>
<td>2.6 ± 2.4</td>
<td>4.0 ± 2.7</td>
<td>8.8 ± 7.0*</td>
<td>7.8 ± 7.4</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. *P < .05. Statistical analysis comparing day 1 to day 0 and days 2 to 7 to day 1 using the Wilcoxon signed rank test.
measurement. This resolved by day 28. Unfortunately, a protocol error precluded the inclusion of patient 1’s salivary cortisol measurements.

Although patient 5’s day 7 salivary cortisol mirrored the suppression shown in her day 7 serum cortisol, interestingly, patient 6’s salivary cortisol levels returned to baseline by day 7, despite residually suppressed serum cortisol. This may be attributed to the fact that the salivary cortisol assay measures free cortisol, whereas the serum cortisol assay measures both free and bound cortisol. Variability or derangement in steroid binding globulins may account for this discrepancy. Alternatively, the difference may also be due to the difference in collection time between saliva (0700 hours) and serum (0800 hours), and subsequent influence of the well-documented diurnal variation in cortisol secretion. A similar duration of effect was seen when studying the effect of intradermal steroid in the 1970s. Meanwhile, intra-articular steroid injections have been shown to have a longer duration of suppressive effect, up to 4 weeks, presumably due to the different pharmacokinetic properties of the joint space and due to the larger doses of steroid injected.

The homogenous response in onset and offset of steroid effect following SILSI would indicate that HPA axis suppression does occur following intralesional steroid but is brief and resolves well before the administration of the next round of SILSI.

A similar effect was observed in immune markers of steroid absorption. An increase in white cell and neutrophil

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**Fig. 2.** Serum cortisol over time. (a) Mean ± SD. (b) Individual participants. PT = patient; SD = standard deviation.

**Fig. 3.** Salivary cortisol over time. (a) Mean ± SD. (b) Individual participants. PT = patient; SD = standard deviation.
counts, and a decrease in eosinophil and lymphocyte counts following steroid administration are known phenomena. Whereas neutrophils are released from bone marrow and undergo delayed apoptosis, lymphocytes and eosinophils are thought to be sequestered in tissues.\textsuperscript{31,32} As expected, mean cell counts mirrored cortisol levels with a noticeable change in levels at day 1 and recovery by day 7 following SILSI.

Meanwhile, no perceptible change in CRP and ESR was observed. Though it is important to be cautious in interpreting the results of this small sample, it is likely that cortisol’s potential effect on these markers is mediated via the suppression of systemic inflammatory disease, and in the absence of such a disease in the context of idiopathic subglottic stenosis, the sensitivity of these tests to detect the effects of glucocorticoid administration may be low.

Further inspection of our daily salivary cortisol data also suggests that triamcinolone is cleared in an exponential, rather than linear fashion. This would indicate that higher doses of steroid would be unlikely to confer particularly longer periods of HPA axis suppression. Of interest, although both patients 4 and 6 received 200 mg of triamcinolone, only patient 6 experienced residual HPA axis suppression at day 7. Conversely, patient 1, who only received 72 mg of triamcinolone was suppressed at day 7. This would indicate that dose may not be the only significant factor affecting systemic effect or glucocorticoid clearance. Of note, the three patients who were suppressed at 1 week following SILSI, patients 1, 5, and 6, were the three oldest patients in our cohort, aged 60, 55, and 61 years. They received 72, 96, and 200 mg of triamcinolone respectively. The three patients who recovered by day 7, patients 2, 3, and 4, were aged 40 to 42 years and received 76, 40, and 200 mg of triamcinolone, respectively. It would therefore seem that older age has a greater association with delayed triamcinolone clearance post-SILSI than dose does. Though our small sample precludes any definitive statements, it is well known that ageing affects not only endogenous cortisol secretion, but also the sensitivity of the HPA axis to change.\textsuperscript{33}

Ultimately, homogenous changes in markers of HPA axis and immune function indicate that steroid absorption does occur, even following intralesional injection of relatively small doses of steroid. However, we have also shown that homeostatic derangement largely resolves by 7 days and well before the time of next injection. Therefore, multiple rounds of SILSI are unlikely to result in side effects associated with sustained supraphysiologic glucocorticoid treatment, such as adrenal insufficiency, Cushing’s syndrome, and immunosuppression. However, it may be appropriate for clinicians to screen at risk patients’ HPA axis function prior to commencing SILSI, and counsel all patients undergoing the procedure on potential short-term and largely unavoidable steroid-related side effects in the first week following the procedure, including appetite change, emotional lability, insomnia, and glycemic derangement. Furthermore, the risk of side effects associated with cumulative lifetime dose of exogenous glucocorticoid should be considered on a case by case basis, balancing the need for SILSI with patient risk factors for avascular necrosis of the hip, osteoporosis, atherosclerosis, fatty liver, or cataracts.\textsuperscript{34,35}

Of note, the tests we performed relay information about systemic absorption, but are less sensitive markers of clinically relevant side effects that might stem from these. To further evaluate possible adrenal insufficiency, dynamic measures of HPA axis function would be required such as an adrenocorticotropic hormone or insulin suppression test. However, these tests are more invasive and are less sensitive indicators of steroid absorption and were therefore not performed during this pilot study.\textsuperscript{36,37}

Overall, our pilot study suggests that serial intrale- sional steroid injection for subglottic stenosis is a safe procedure, with minimal, transient risks; however, clinicians should assess each patient individually and consider risk factors for both acute steroid-related side effects, as well as longer-term, insidious side effects associated with a cumulative lifetime dose of glucocorticoid.\textsuperscript{38,39,40}

Evaluating our protocol, it would appear that 0, 1, and 7 days were appropriate time points to capture a baseline, peak effect and return to normal while at the same time minimizing patient burden. Daily salivary testing allowed for further characterization of HPA axis function in the first week following SILSI, demonstrating that resolution may in fact occur at 5 to 6 days. The tests selected appear to be sufficiently sensitive to detect changes in physiological function. Future research may address limitations in the design of this pilot study.

A key analytical issue was the small sample size. SGS is a rare disease, and of those treated in our center, an even smaller group receive SILSI alone. We decided not to include patients undergoing endoscopic intervention with adjuvant steroid injection, as surgery usually involves de-epithelialization of the subglottis, which may result in a different rate of retention and absorption. Additionally, the physiological stress of undergoing the procedure awake versus under general anesthetic may affect circulating cortisol levels.
A small sample, compounded by diurnal variation in cortisol secretion, as well as day-to-day inter- and intra-subject variability in circulation cortisol, resulted in a degree of variability in measures of HPA axis. In addition, we collected overnight urine samples to measure overnight creatinine-adjusted urinary cortisol. This would have served as an integrated measure of HPA axis function. Some subjects collected the samples incorrectly, however, resulting in significant heterogeneity in the sample. Therefore, we chose not to include these results in our analysis. Nonetheless, we have observed a significant and largely homogenous change in other markers of HPA axis function over time.

Although it appears that systemic effects resolve well before the administration of the next dose of steroid, we would like to confirm this by assessing intrasubject variation in systemic biomarkers over multiple rounds of SILSI. Though unlikely, this would serve to characterize any suboptimal function of the pituitary—adrenal axis over time.

CONCLUSION

Our study suggests that patients undergoing SILSI experience peak systemic effects 1 day following their procedure, with restoration of homeostasis occurring at 1 week or shortly thereafter, well before the next injection. This suggests that the spacing of injections by 3 to 5 weeks, as proposed by Franco et al., is appropriate and will not result in side effects associated with prolonged supraphysiologic doses of glucocorticoid. Practitioners performing SILSI should nonetheless consider short- and long-term side effects that may occur with any systemic steroid administration and discuss these with at-risk patients on a case-by-case basis.

BIBLIOGRAPHY