



Diurnal variations in subcutaneous allergen immunotherapy reactions

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ARTICLE INFO

Article history:

Received for publication July 28, 2016.

Received in revised form September 23, 2016.

Accepted for publication October 10, 2016.

ABSTRACT

Background: Circadian rhythms underlie many immune responses and allergic diseases. Subcutaneous immunotherapy (SCIT) can result in adverse reactions; however, it is unclear whether such reactions have a diurnal pattern.

Objective: To assess whether the timing of SCIT affects the rate of adverse reactions.

Methods: This study was a retrospective medical record review of adult patients (n = 289) who received SCIT at the Northwestern Medical Faculty Foundation, Chicago, Illinois, during a 10-year period (2004–2014). Injections were given in the outpatient setting. There were a total of 17,457 injections with 574 reactions. Covariates included age, sex, median income, asthma status, vial contents, number of injections, and previous immunotherapy reactions. Logistical regression was used to calculate the odds of having a reaction with time of SCIT administration as the primary determinate.

Results: Immunotherapy reactions occurred more frequently after afternoon or evening (PM) injections (328/8721 = 3.8%) vs morning (AM) injections (246/8736 = 2.8%), ($\chi^2 = 12.26$, $P < .01$). Systemic reactions, defined as World Allergy Organization grade 1 or higher, did not have diurnal variation (59/8721 = 0.67% for PM vs AM 56/8736 = 0.64% for morning; $\chi^2 = 0.08$; $P = .77$). PM injections resulted in higher odds of reaction compared with AM injection in a fully adjusted logistic regression model (odds ratio = 1.43; 95% confidence interval, 1.20–1.70; $P < .01$). When considering time as 4 categories, the highest odds of reaction were noted for the period from 15:01 to 17:30 (odds ratio, 1.55; 95% confidence interval, 1.21–2.00; $P < .01$).

Conclusion: PM injections of SCIT are associated with increased cutaneous reaction rates when compared with AM injections. In patients experiencing bothersome local reactions, it may be beneficial to administer SCIT in the morning.

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Introduction

Subcutaneous immunotherapy (SCIT) is an effective therapy for allergic rhinitis, asthma, and stinging-insect hypersensitivity.¹ Given that patients are being injected with a known allergen, reactions are expected. Overall reaction rates vary, with approximately 30% to 50% of patients experiencing a reaction.^{2,3} Systemic reactions occur at a rate of approximately 0.1% to 1% per injection.^{4–6} Local reactions are significant in that they may be perturbing to the patient, may delay dose advancement, and may cause patients to discontinue treatment prematurely; in short, large local reactions incur significant costs for the patient and health care system.⁷

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<http://dx.doi.org/10.1016/j.janai.2016.10.007>

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Previous research on diurnal variations in SCIT reaction rates is limited to one abstract that assessed systemic reactions. In a retrospective medical record review, Boleman et al⁸ reported no association between rate of immunotherapy reactions to the time of day or stage of lunar cycle. *Middleton's Allergy: Principle and Practice* textbook asserts that “the lack of effect of the circadian cycle on the incidence has been reported”⁹; however, there data are limited to support this claim.

Although not clinically significant,^{10,11} it is established that cutaneous hypersensitivity reactions are smallest at approximately 9 AM, with larger wheal sizes to skin prick testing in the late morning or afternoon.^{12,13} This pattern of cutaneous hypersensitivity corresponds to endogenous cortisol peak shortly after awakening, followed by decreased levels in the afternoon, with an overnight nadir.¹⁴ Applying what is known about skin and immunologic circadian rhythm¹⁵ could improve the safety of immunotherapy by simply changing the time of SCIT injection.

Our objective was to perform a retrospective medical record review on the robust experience with SCIT at the Northwestern Medical Faculty Foundation, Chicago, Illinois, to explore the frequency of both systemic and local reactions to immunotherapy, depending on the time of day administered. Considering the diurnal immunologic and corticosteroid rhythms that affect skin testing response and systemic markers of inflammation, our hypothesis was that patients receiving immunotherapy are more likely to have reactions in the afternoon or evening (PM) compared with the morning (AM).

Methods

A retrospective medical record review of all patients ($n = 289$) who had received SCIT at the Northwestern Medical Faculty Foundation during a 10-year period (2004–2014) was performed. This study was approved by the Northwestern University Institutional Review Board. All patients were identified through a paper record of their immunotherapy that was stored in a locked drawer. No patients were purposefully excluded. It is possible that some patients who underwent SCIT had missing records. SCIT was administered by nurses who received standardized training in the recognition and treatment of reactions. Nurses work full days, so there was no variation in nurses administering and assessing AM VS PM reactions.

A reaction was defined as any adverse event subjectively or objectively experienced by the patient after receiving their SCIT. Reactions were defined as systemic or local. Systemic reactions were those that met the criteria for a score of 1 or higher based on the World Allergy Organization Subcutaneous Immunotherapy Systemic Reaction Grading System.¹ All systemic reactions were scored on a scale of 1 to 4 based on this scoring system. Per this system, diffuse pruritus alone counted as a grade 1 systemic reaction. Reactions that did not meet the aforementioned criteria were categorized as local reactions. For example, if only a few hives around the injection site were noted, this was considered a nonsystemic reaction. Local reactions were subcategorized as large local reactions if size was 25 mm or greater.²

Local reaction sizes and characteristics (induration, erythema) were recorded in the paper record, along with the patient's description of the reaction. Because several physicians in the group dose adjust after large local reactions, measurements for all cutaneous reactions are generally recorded. For systemic reactions, each patient's symptoms and use of epinephrine or other medications were documented. SCIT was prescribed by 6 physicians at the Northwestern Medical Faculty Foundation, and all injections took place in the same outpatient facility. All physicians follow a specified formula in terms of allergen immunotherapy prescription. They follow the same protocol in selecting antigens based on results of immediate hypersensitivity skin prick or intradermal testing. Tree and grass immunotherapy is administered as a standardized mix of locally relevant allergens.

The time of each injection administered was organized into 4 periods for data collection (7:30 to 10:00, 10:01 to 12:30, 12:31 to 15:00, and 15:01 to 17:30). AM was defined as 7:30 to 12:30, whereas PM was defined as 12:31 to 17:30. SCIT was rarely given during the lunch hour, which was from noon until 13:00.

Patient and Reaction Characteristics

Individual patient characteristics were collected for each patient: age, sex, history of asthma (yes or no), allergens administered, timing of injection, total number of injections, and number of reactions. Median household income was also estimated using data from the patient's zip code. The following characteristics of individual reactions were collected as well: time injection given, date given, strength of injection given (maintenance vs buildup), time between injection and onset of reaction, duration of reaction, date

of prior injection, history of previous reaction, pretreatment before injection, medication given after injection, if reaction took place during allergy season, and size of reaction. Injections were defined as buildup if strength given was below maintenance dose. Reactions were characterized as systemic or local cutaneous only. There were some local reactions in which the exact size was not recorded. In addition, some reactions occurred after the 30-minute observation period and were noted in the medical record at the follow-up visit. These reactions were included in our data set and analysis.

Determinant and Key Variables

The primary determinant was time of injection, which was coded as described above. For data analysis, we grouped injection timing into AM (7:30 to 12:30) or PM (12:31 to 17:30) to determine whether there was a difference in reaction rates for AM VS PM. There was no difference across the compressed groups, and this method was chosen a priori to increase power in our regression model. Covariates were chosen because they have been reported in previous literature to be known risk factors for reactions to immunotherapy.^{16,17} Selected covariates included receiving Hymenoptera immunotherapy, previous reaction to SCIT, and history of asthma. Other standard covariates included were age, sex, and median income (as a reflection of socioeconomic status). Other covariates, such as time lapsed since last immunotherapy, use of premedication before receiving immunotherapy, and administration of immunotherapy during allergy season, were not available in both reactors and nonreactors; therefore, these covariates were not included in the logistic regression model.

Statistical Analysis

Descriptive statistics, including χ^2 analysis and t tests, were used to compare patient characteristics of reactors vs nonreactors and reaction rates and characteristics.

We then constructed a logistic regression model to determine whether the association between timing of SCIT administration and adverse reactions would remain significant when controlling for relevant covariates. The primary outcome was a binary variable of whether or not the adverse reaction was caused by the SCIT injection (yes or no). The primary determinant was time of injection (AM VS PM or defined by 1 of the 4 grouped injection timing).

Results

Patient Characteristics

A total of 289 patients were included in this retrospective medical record review; 156 were reactors, and 133 were nonreactors, with no deaths. No significant difference was found between reactors and

Table 1

Characteristics of Patients Undergoing Subcutaneous Immunotherapy (SCIT), 2004–2014: Reactors vs Nonreactors^a

Characteristic	Overall (N = 289)	Reactor (n = 156)	Nonreactor (n = 133)	P value ^b
Age, mean (SD), y	43.0 (11.9)	43.3 (11.6)	42.7 (12.3)	.65
Male	126 (43.6)	49 (31.4)	77 (57.9)	<.01
Asthma diagnosis	156 (54.0)	87 (55.8)	69 (51.9)	.51
Receiving Hymenoptera SCIT	16 (5.5)	11 (7.1)	5 (3.8)	.30
Receiving pollen-only SCIT	134 (46.4)	75 (48.0)	59 (44.3)	.53
Total No. of injections, mean (SD)	60.4 (40.4)	67.4 (39.0)	52.2 (40.7)	<.01
Income by zip code, median (SD), \$	72,290 (26,263)	69,915 (25,684)	75,075 (26,754)	.10

^aData are presented as number (percentage) of patients unless otherwise indicated.

^bComparing reactors vs nonreactors.

Table 2
Subcutaneous Immunotherapy Frequency of Reactions by Time

Time	No. (%) of reactions				χ^2	P value
	7:30–10:00 (n = 6,097)	10:01–12:30 (n = 2,639)	12:31–15:00 (n = 6,210)	15:01–17:30 (n = 2,511)		
Local cutaneous reactions ^a	135 (2.2)	55 (2.1)	177 (2.9)	92 (3.7)	$\chi^2 = 18.85$	$\leq .01$
Systemic reactions ^b	42 (0.7)	14 (0.5)	40 (0.6)	19 (0.7)	$\chi^2 = 1.14$	.77
Total reactions	177 (2.9)	69 (2.6)	217 (3.3)	111 (4.4)	$\chi^2 = 17.56$	$\leq .01$

^aAll nonsystemic cutaneous reactions.^bAll systemic reactions per the World Allergy Organization criteria.

nonreactors in terms of age, asthma status, and median income (Table 1). Reactors were more likely to be female (107/156 = 73%) vs nonreactors (56/133 = 42%, $P < .01$). Reactors cumulatively during their immunotherapy had received more injections than nonreactors (mean, 67 vs 52; $P < .01$) (Table 1). Females received more injections than males (mean, 63 vs 57; $P < .01$).

Reaction Rates vs Bivariate Time

Overall, there were 17,457 immunotherapy injections retrospectively analyzed. Of these, 8,736 were given in AM vs 8,721 given in PM. Frequency of injections by time and reactions (local cutaneous vs systemic) are presented in Table 2, with rates of reactions depicted in Figure 1. Overall, SCIT reactions occurred more frequently after PM injection (328/8,721 = 3.8%) vs AM (246/8,736 = 2.8%) ($\chi^2 = 12.26$, $P < .01$). Although overall reactions more commonly occurred after PM injection, systemic reactions did not have diurnal variation (59/8,721 = 0.67% for PM vs 56/8,736 = 0.64% for AM; $\chi^2 = 0.08$; $P = .77$). Local cutaneous reactions were found to occur more frequently after PM injections (269/8,721 = 3.1%) vs AM (190/8,736 = 2.2%) ($\chi^2 = 14.10$, $P < .01$). Considering extremes of time, injections after 15:00 were more likely to result in local

cutaneous reaction (92/25,11 = 3.7%) compared with before 10:00 (135/6,097 = 2.2%) ($\chi^2 = 14.56$, $P < .01$).

Characteristics of Reactions

A total of 574 reactions (246 AM and 328 PM) were included in this study, of which 193 AM and 266 PM injections were local cutaneous only. Characteristics described include maintenance status, World Allergy Organization score, size of local reaction, type of allergen injected, number of injections, and season of injection (Table 3). The rate of grade 1 World Allergy Organization reactions did not significantly differ in PM (40/8,721 = 0.46%) vs AM (30/8,736 = 0.34%) injections ($\chi^2 = 1.45$, $P = .23$). Data on size of local reactions were recorded in 418 injections, with size of local reactions not significantly different in the PM (mean, 49.1 mm) vs AM (mean, 47.6 mm) ($P = .76$). Among reactions, no significant difference was found in the proportion of patients undergoing maintenance therapy in the PM (142/246 = 57.7%) vs AM (199/328 = 60.7%) ($\chi^2 = 0.51$, $P = .48$). Perennial allergens were more likely to result in PM reactions, whereas nonperennial allergens were more likely to result in AM reactions.

Logistic Regression Model

PM injections of SCIT were more likely to result in reaction compared with AM injections in fully adjusted models (odds ratio [OR], 1.43; 95% confidence interval [CI], 1.20–1.70; $P < .01$) (Table 4). When considering time as 4 categories, the highest odds of reaction were noted for the period from 15:01 to 17:30 (OR, 1.55; 95% CI, 1.21–2.00; $P < .01$) (Table 4).

Discussion

This retrospective medical record review was performed to determine the frequency of reactions to immunotherapy depending on time of day administered. We found that patients receiving SCIT in the PM were more likely to have reactions than those receiving AM injections. This finding was significant for local cutaneous reactions but not systemic reactions. Our finding of diurnal

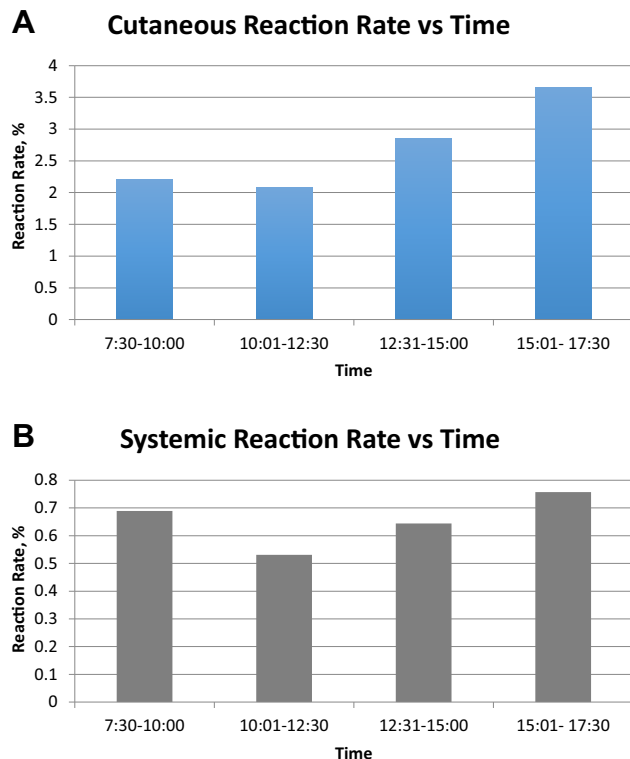


Figure 1. Cutaneous (A) and systemic (B) reaction rates to subcutaneous immunotherapy based on time of day administered, with rate as a percentage of all injections during that period resulting in a reaction.

Table 3
Characteristics of AM vs PM Reactions^a

Characteristic	AM	PM	P value
Local cutaneous reactions	193	266	NA
Large local cutaneous reactions ^b	124	172	NA
Total reactions	246	328	NA
Maintenance injection	142 (57.7)	199 (60.7)	.48
Perennial allergen reaction	64 (26.0)	117 (35.7)	.01
Nonperennial allergen reaction	182 (74.0)	211 (64.3)	.01
Reaction during allergy season (nonperennial injection reactions)	85 (46.7)	79 (37.4)	.03
Size of all local reactions, mean (SD), mm (n = 418)	47.6 (46.2)	49.1 (54.0)	.76
Size of large local reactions, mean (SD), mm ^b (n = 296)	62.5 (45.6)	67.1.0 (55.4)	.45

Abbreviation: NA, not applicable.

^aData are presented as number (percentage) of reactions unless otherwise indicated.^bLarge local cutaneous reactions defined as any skin reaction 25 mm or larger.

Table 4
Odds of Reaction to Subcutaneous Immunotherapy and Time of Injection

Time of injection	Unadjusted OR (95% CI)	Adjusted OR ^a (95% CI)	P value
AM (n = 8,736)	1.0 [Reference]	1.0 [Reference]	
PM (n = 8,721)	1.35 (1.14–1.60)	1.43 (1.20–1.70)	<.01
7:30–10:00 (n = 6,097)	1.0 [Reference]	1.0 [Reference]	
10:01–12:30 (n = 2,639)	0.90 (0.68–1.19)	1.01 (0.76–1.35)	.99
12:31–15:00 (n = 6,210)	1.21 (0.99–1.48)	1.38 (1.12–1.71)	<.01
15:01–17:30 (n = 2,511)	1.55 (1.21–1.97)	1.55 (1.21–2.00)	<.01

Abbreviations: CI, confidence interval; OR, odds ratio.

^aAdjusted for age, sex, median income, history of asthma, receiving Hymenoptera immunotherapy, receiving pollen-only immunotherapy, number of injections, previous and reaction to subcutaneous immunotherapy.

variation in local cutaneous reactions, with greater reaction rates in the PM, is consistent with literature reporting larger skin test reactions in the PM.^{13,15} Similar to previous studies, although we found increased wheal size of cutaneous reactions in the PM VS AM, differences were not statistically significant.

Diurnal variation in skin responsiveness is related to both cortisol rhythm and other circadian properties.¹⁵ Specifically, serum cortisol generally peaks between 8 and 9 AM, resulting in a smaller wheal size, with upregulated allergen responses in the PM.¹⁸ These circadian properties hold true for SCIT reactions, where afternoon reactivity is higher, resulting in a higher rate of cutaneous reactions. Given that rates of reaction are lowest in the AM and highest in the PM, specifically after 3:00 PM, it may be beneficial for patients who are bothered by local reactions to receive SCIT in the morning. This is a safe, inexpensive, and simple measure that can be taken to help reduce reactions and improve the safety of SCIT for a select number of patients.

One of the major strengths of this study is the size of study (17,487 total injections) and the amount of data that were collected for each patient and reaction. Furthermore, all SCIT was given and data were collected at the same office by the same medical team, which provided consistency in our data with regard to injection technique and limited variation with reaction documentation and treatment. In addition, our injection hours are standard, and we do not give injections during the weekends. The robust reaction data allowed for a logistic regression model with control of key covariates. Even when these covariates were controlled for, we found that there was a significant difference in the odds of having a reaction in the PM VS the AM.

This study has several limitations. Given that this was a retrospective medical record review, there are limitations to extrapolating findings compared with prospective studies. There were 8 instances when patients reported general pruritus after the 30-minute observation period. These reactions in accordance to World Health Organization guidelines were categorized as systemic. In addition, it is possible that very small local reactions were sometimes not documented. Although we did not find any diurnal variation in systemic reactions, this is likely because we were not powered to see such a low frequency event. However, even if we were powered to see a significant difference between AM VS PM systemic reaction rates, clinically, the number needed to treat would be very high for this difference to be relevant. Thus, timing of injection is not an important variable to control in preventing systemic reactions.

Furthermore, the dividing of time of injection into 4 periods was chosen based on our clinic schedule, which could be different than other practice schedules. Although it is well established that altered sleep patterns affect cortisol levels¹⁹ and antigen reactivity,²⁰ this study did not take into account sleep schedules or cortisol levels. It is possible that some patients were shift workers and that this affected their sleep and circadian rhythm. Other variables unaccounted for include menstrual cycle, whether or not antihistamine

was taken consistently, and asthma only vs asthma and allergic rhinitis. Environmental factors, such as pollen counts, change depending on the time of day.^{21,22} We were unable to account for this variable. Although several physicians recommend PM injection of antihistamines, the use of preinjection antihistamine, type of antihistamine, and timing of antihistamine pretreatment before immunotherapy were not consistently recorded and could not be included in our model, which could influence our findings. Other possibly pertinent covariates that were excluded in our regression model because of lack of data in nonreactors included season and prior skin test reactivity. We did have these data for reactors and included these data in reported reaction comparisons.

Diurnal variations are tied to serum cortisol and other circadian factors, which are likely different among patients, and explain why previous studies found some patients with larger AM to PM difference in wheal size compared with other patients who had very little difference.¹³ However, these differences in mast cells are not large enough to alter skin prick test size significantly, as demonstrated in our cohort and in others.^{10,11} Future studies should evaluate prospectively in a larger cohort whether AM injections of SCIT decrease reaction rates. Given the high frequency of local reactions in sublingual immunotherapy, future work might also explore whether AM administration of sublingual immunotherapy can decrease adverse events.

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