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Research Article

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Dose Dependent Urine Concentrations of Gabapentin (Neurontin®)

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Abstract

Gabapentin (Neurontin®) is frequently prescribed for a number of conditions including adjunctive therapy for partial seizures and neuropathic pain. Gabapentin is unique to most drugs in that it is titrated quickly to high doses (1,800-3,600mg/day or greater) due to its low toxicity. It is not metabolized but excreted primarily unchanged in the urine at extremely high levels ranging from 5µg/ml to >30,000µg/ml. The work reported here looks at gabapentin urine drug testing (UDT) results from 6 months of clinical urine specimens in which gabapentin was detected (n=35,526), prescribed (n=23,432, 66%) or not prescribed (n=12,094, 34%). In the prescribed population, gabapentin was primarily prescribed to females (61%). The overall age for positive results ranged from 14 to 97 years with an average age of 56.5 years. Interestingly, the average age of those patients positive for gabapentin without a prescription and positive for any illicit was 42.7 years. These data indicate that at a maximum, 34% of the total gabapentin positive samples are from abuse (no prescription). Attempts at normalization and transformation of drug concentration data using creatinine normalization did lead to a near Gaussian distribution where +/- 3 standard deviations may be estimated. It remains difficult to determine if a patient is abusing the drug when the UDT values are extremely high for patients prescribed gabapentin.

Keywords: Dose ranges, Gabapentin, LC-MSMS and Toxicology

Introduction

Gabapentin (Neurontin®) was approved for use as an adjunctive anticonvulsant in 1994, but has since been used for other conditions (e.g. neuropathic pain, fibromyalgia) and is commonly co-dosed with other therapeutics for chronic pain [1]. It is a unique drug as it is not metabolized, has a short half-life, and is excreted primarily unchanged in the urine at very high concentrations. Dosing can be as high as 3,600-4,800mg/day without toxic impact [2]. However, there is growing concern over the potential for abuse and misuse of gabapentin, especially since it is showing up in polydrug impaired driving and postmortem overdose cases (with other drugs). It has been reported to potentiate the effects of methadone and has been combined with antipsychotics (e.g. quetiapine) to achieve a weak "cocaine-like" high [3-10].

Gabapentin is not in the top 100 prescribed drugs, but brought in annual sales as high as $3 billion in 2003. The number of prescriptions increased ~42% in only 4 years (2011-2015). This large increase in prescriptions has brought about concerns for abuse and misuse. Misuse in the general population is reported to be approximately 1%, and as high as 40-65% among those with prescriptions [3]. A review of cases submitted for impaired driving to the Washington State Toxicology Laboratory between January 2002 and December 2007 showed that 93% of gabapentin positives indicated polydrug use [5]. Gabapentin abuse is high in prison populations and in patients who are taking opiates; either for chronic pain or for illicit use [3,6-8]. As such, it is becoming more important for physicians to monitor patients for diversion (e.g. selling or giving their prescriptions to other individuals) or for abuse.

The goal of this work is to present the results of a significant number of patient urine tests for gabapentin as raw, unmodified data. These data are examined with respect to dose, age, sex, and whether or not these data alone would enable physicians to determine potential adherence to the patient's dosing paradigm. Mathematical transformation of these data using patient creatinine is also examined

in an attempt to further define a "normal" population which can be differentiated from non-adherent/drug abuse populations.

Materials and Methods

Standards were purchased from Cerilliant Corporation (Round Rock, TX) as 1mg/mL stock solutions. An enzyme solution was prepared by diluting IMCSzyme® β-glucuronidase solution (IMCS, Irmo, SC) to 10,000 units/mL in 0.02 M sodium phosphate buffer, pH 7.5. Inasmuch as the analysis of gabapentin was part of a larger method, a hydrolysis control was prepared by making a stock of 2.5 ng/mL of morphine-3β-D glucuronide in normal human urine [11]. This control is run with every batch to verify proper hydrolysis of all samples. Further details about this method and validation thereof are available in an earlier report [11].

LC-MS/MS Method

The LC-MS/MS method detailed herein was originally performed on an Agilent LC/MSMS 6460 system [11]. The current version of this method runs on a Thermo HPLC/MSMS system using an Ultra MSMS unit. This necessitated several changes from the original method. For example, solvents A (5mM ammonium formate with 0.1% formic acid [aqueous]) and B (5mM ammonium formate in 75:25 methanol: acetonitrile with 0.1% formic acid) were used to provide a gradient. A flow rate of 800µl/min was used throughout. Total cycle time was roughly 6.5 min. A Phenomenex (Torrance, CA) Kinetex 2.6µm Phenyl-Hexyl 100Å, 50 x 4.6mm (00B-4495-E0) HPLC column was used in this method similarly to the original method. The injection volume was set to 15µL and column temperature was set to 30°C. Specific analyte transitions and internal standard assignments are given in the earlier report [11].

As mentioned, the LC/MSMS method is a much larger method testing for 34 analytes using 15 internal standard compounds in a single injection. The specifics of this method and the validation data are summarized in an earlier report [11]. Validation results for gabapentin are summarized as followed. The limit of detection (LOD)/limit of quantitation (LOQ) was established at 1µg/mL and the upper limit of linearity (ULOL) was established at 500µg/mL. The average carryover seen after injecting samples spiked at the ULOL was < 0.05µg/mL. Three different concentrations, 2µg/mL, 15µg/mL and 20µg/mL were tested over a 3 day period for precision and accuracy with resulting percent target values of 99.6%, 96.8% and 98.5% and percent CVs of 4.6%, 4.4% and 6.1% respectfully. Matrix effect was determined at -2.34% and there were no interfering compounds identified. Compounds tested for interference are available in the earlier report [11].

Data Analysis

The results for gabapentin from this method were "curated" as follows in an attempt to determine an adherent population.

1. Include patients who were prescribed and tested positive for gabapentin.
2. Patients positive for any illicits were not included.
3. Patients not consistent with other prescriptions were not included.
4. Patients who failed sample validity testing (e.g. pH, creatinine and specific gravity) were not included.
5. Samples returned as >500.0µg/mL were not included.

The data analysis and model development were conducted using R Project version 3.3, a language and environment for statistical computing and graphing. Data smoothing was conducted by kernel density estimation, which is a well-accepted mathematical tool to smooth continuous data (e.g. histograms) [12]. While model development detailed in earlier reports [13-15] resulted in equation 1.

$$NORM_{D_{CONC}} = ln\left(\frac{Aconc * LBW * Age * pH}{D_{DOSE} * CREAT}\right) \quad (1)$$

Where *ln* is the natural log, Aconc is the concentration of the measured analyte in kg/L; LBW is the lean body weight of the subject in kg; Age is the subject age in years; pH is the sample fluid pH; DDOSE is the subject prescribed drug dosage in kg/day; and CREAT is the sample fluid creatinine concentration in kg/L, the work herein attempted to identify a "normal" population with as little mathematical manipulation as possible.

Thus the following equation was used in this work for patients prescribed gabapentin:

$$NORM_{D_{CONC}} = ln\left(\frac{Aconc}{CREAT}\right) \quad (2)$$

To recenter the resulting near Gaussian curve, the value of was modified by subtracting the mean of the data set and dividing by the standard deviation as shown here:

$$Zscore = (NORMDdose - mean)/(Standard\ Deviation) \quad (3)$$

The mean of the values of was determined to be -1.120 and the standard deviation was 1.090. The result, the standardized normal value, is shown in Figure 3.

Statistical analysis for comparison of group mean values was performed by using non-parametric Mann Whitney test in Graph Pad Prism.

Results

Here, 6 months of urine test results were examined for this study, a total of 298,554 samples. Of the total number of gabapentin positive samples (35,528), 23,433 were from patients prescribed the drug while another 12,094 samples were derived from patients without gabapentin prescriptions. These numbers indicate that a maximum of 34% of the total population of 35,528 patients is potentially abusing gabapentin not including patients prescribed the drug. The average age of patients prescribed gabapentin and positive for gabapentin is 56.5 years. Those patients positive for gabapentin and without a prescription averaged 42.7 years for those with an illicit present and 52.4 years for those without a positive illicit result, much closer to the positive group with a prescription. A Mann Whitney test was used to compare the median values of those positive with a prescription (300.6µg/mL) and those positive without a prescription (244.2µg/mL) and showed that the groups are statistically different with a P

value < 0.0001. Any differences between sexes were not statistically significantly different.

Patient samples positive for gabapentin without a prescription were examined to see if and what additional drugs might be present for those patients. Figure 1 shows the relative frequency of each test class for these samples with 18.3% taking a benzodiazepine, 12.3% taking an opiate, 4.7% taking oxycodone/oxymorphone, amphetamine at 5.6% and buprenorphine at 5.2%.

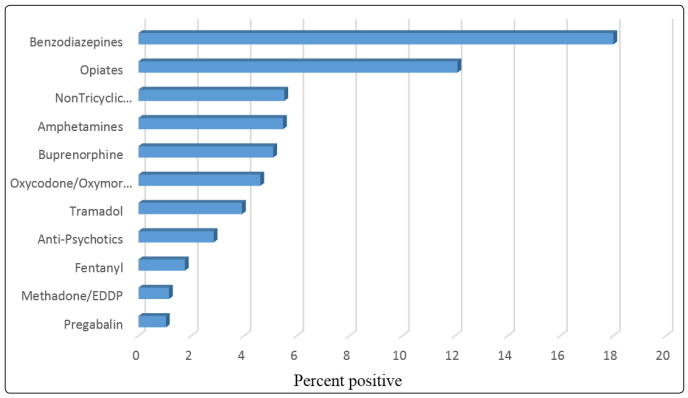

Figure 1: Non-prescribed drugs in patients positive for gabapentin without prescription

Figure 2 shows the median, the first and third quartiles (i.e. 25% and 75% of the data respectively) and the range between 5% and 95% of the total cleaned data set (14490 patients cleaned from 23,433 total prescribed patients) by dose/day. Figure 2 also provides the number of samples within each dose group on the right which vary considerably from 16 patients at 700mg/day to 2424 patients at 900 mg/day. These parameters are listed in Table 1 for each daily dose up to 3600mg/day. Doses at and above 2400mg/day are not statistically significantly different as shown in Table 2. This is also apparent from Figure 2 where the Median values do not differ for doses above 2400mg/day. Thus above this dose level, the median urine concentration appears to be independent of dose. Approximately 61% of these patients are female. This does not change with dose.

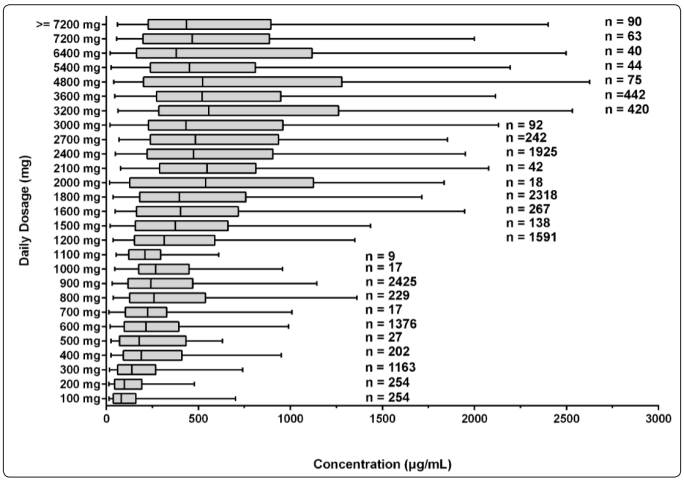

Figure 2: Graphical representation of cleaned gabapentin data with dose and number of specimens

Table 1: Descriptive statistics

Dose / Day	100 mg	200 mg	300 mg	400 mg	600 mg	800 mg	900 mg	1200 mg	1500 mg	1600 mg	1800 mg	2400 mg	2700 mg	3200 mg	3600 mg
n=	254	226	1163	202	1376	229	2425	1591	138	267	2318	1925	242	420	442
Minimum	5.641	5.274	5.046	5.399	5.146	9.387	5.178	5.181	8.000	5.808	5.336	5.367	6.251	6.233	5.161
25% Percentile	38.599	44.521	61.789	93.505	97.422	126.934	118.715	153.916	157.571	164.157	181.251	222.227	239.266	285.171	272.543
Median	81.843	96.726	139.142	189.718	215.431	258.846	242.447	315.272	375.890	403.517	396.489	473.550	483.613	556.480	520.857
75% Percentile	160.214	184.945	268.795	410.326	393.741	538.193	469.928	589.780	661.009	716.953	758.433	904.442	935.823	1262.000	948.045
Maximum	4186	691	14940	2106	9430	3232	27930	15880	2292	12280	18930	17650	3829	5752	8424
5% Percentile	15.620	14.572	16.585	24.817	21.155	38.179	32.845	36.141	20.534	48.361	37.097	49.886	69.486	63.002	46.408
95% Percentile	702.255	480.817	741.210	950.523	990.406	1362	1144	1350	1436	1947	1714	1951	1853	2531	2114
Mean	204.149	141.324	241.004	296.782	322.283	413.753	382.455	461.599	479.229	652.149	600.410	690.197	676.590	863.874	770.001
Std. Deviation	449.669	138.133	540.768	307.594	439.154	471.534	704.335	602.169	438.363	1024.000	843.596	809.353	633.553	817.454	848.321
Std. Error of Mean	28.215	8.667	15.857	21.642	11.839	31.160	14.303	15.097	37.316	62.692	17.522	18.447	40.726	39.888	40.351
Lower 95% CI of mean	148.584	124.255	209.893	254.107	299.059	352.355	354.408	431.988	405.439	528.715	566.050	654.019	596.364	785.468	690.698
Upper 95% CI of mean	259.715	158.393	272.116	339.457	345.508	475.151	410.503	491.211	553.019	775.583	634.769	726.375	756.816	942.279	849.304

concentrations in µg/mL

Dunn's Multiple Comparison

100 mg														
200 mg	No													
300 mg	No	Yes												
400 mg	Yes	Yes	No											
500 mg	No	No	No	No										
600 mg	Yes	Yes	Yes	No										
700 mg	No	No	No	No	No									
800 mg	Yes	Yes	Yes	No	No									
900 mg	Yes	Yes	Yes	No	Yes	No								
1000 mg	No	No	No	No	No	No	No							
1100 mg	No	No	No	No	No	No	No							
1200 mg	Yes	Yes	Yes	Yes	Yes	No	Yes							
1500 mg	Yes	Yes	Yes	Yes	Yes	No	No	No						
1600 mg	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No					
1800 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No				
2000 mg	Yes	Yes	Yes	No	No	No	No	No	No	No	No			
2100 mg	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No			
2400 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes			
2700 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No		
3000 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	
3200 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	
3600 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No
4800 mg	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	No	No	No
5400 mg	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No	No
6400 mg	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No	No	No	No
7200 mg	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No	No
>= 7200 mg	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	No	No	No
Dose/Day	100 mg	200 mg	300 mg	400 mg	600 mg	800 mg	900 mg	1200 mg	1500 mg	1600 mg	1800 mg	2400 mg	2700 mg	3200 mg	3600 mg

Statistically Significant Difference?

Normalization and transformation of the positive prescribed data is shown in Figure 3. The features of this graph suggest a Gaussian or near Gaussian distribution. The distribution has been re-centered to a mean of 0 and a standard deviation of 1 to compare with a true Gaussian distribution which illustrates the differences. The implications of this distribution are discussed below.

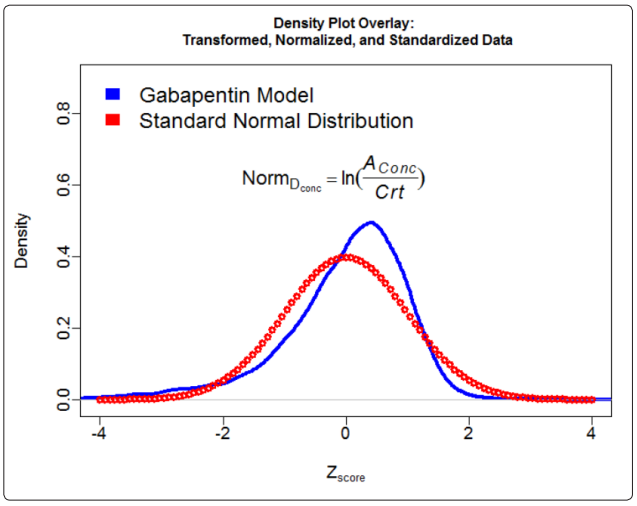

Figure 3: Kernel density estimation plot of normalized data (see methods for details).

Discussion

Urine Drug Testing (UDT) is often used to help assess a patient's adherence to their prescribed medication [16]. When UDT results are returned to the physician, they can generally determine whether the patient is positive or negative for the drug in question. But questions can remain, for example, is a low value (e.g. 0.500µg/mL) positive for gabapentin? If so, is it clinically relevant? Is the patient taking their medication as prescribed or on an 'as needed' basis? Are they taking a small amount and selling the rest; e.g. diverting their medication? Similarly, is an extraordinarily large amount of drug in urine the result of taking the drug "as needed"? Or is it just a reflection of "pill scraping" into the urine cup in an attempt to escape detection (of diversion)?

Heltsey, et al. reported on the "prevalence" of gabapentin in a general pain population indicating that 12.2% of the population was positive for gabapentin using an LOQ of 2.5µg/mL and a ULOL of 1,000.0µg/mL [17]. They also found that 249/57,542 were positive for both gabapentin and pregabalin (0.4%). Our data indicate that pregabalin is present at 1.0% of gabapentin positives without prescription (Figure 1). The range of gabapentin concentrations determined in their study was from 2.5µg/mL to 35,345µg/mL with a mean concentration of 430.9µg/mL. Their data were averaged across all doses and thus returned a lower mean concentration than that observed for the higher doses in this work. The overall prevalence result from this work was 11.9%, very close to that observed in the earlier work. This includes both prescribed and/or positive patients. Using only those prescribed the drug, the prevalence is 7.8%.

Gabapentin is different from most other drugs. For example, at a dose of 100mg/day, the median urine concentration was 81.843µg/mL with a minimum value of 5.641µg/mL (Figure 2, Table 1). Even at lower doses, gabapentin concentration in urine is very high relative to other pain medications and certainly elicits where reporting cut-offs of 2ng/mL or less (e.g. fentanyl, buprenorphine

etc.) are common. Results between total daily dose levels often do not differ significantly as shown in Table 2. As shown in Figure 2, it would be difficult if not impossible to determine whether a patient had taken the prescribed dose if that dose was over 2,000mg/day. From Figure 2, it is readily seen that doses at and above that level are common. Only doses below 1,500mg/day could possibly be differentiated from higher doses by using the raw UDT data. In short, the raw data are generally only useful for determining positive or negative for gabapentin.

Figure 3 shows an attempt to normalize the raw gabapentin data to patient creatinine values [13,18]. The reported concentration divided by the respective creatinine concentration is then transformed via a logarithmic function to afford the result seen in Figure 3 (Equation 2). The Zscore (i.e., the standardized normal value) is then calculated by subtracting the mean and dividing by the standard deviation for comparison with a theoretical Gaussian Curve. As reported in other modelling work, this near Gaussian distribution can be used to estimate which patient results are part of a population of adherent patients and those that may be suspect. Values outside +/- 3 standard distributions from the mean in a Gaussian distribution have a<1% probability of being part of the "normal" population. Nevertheless, gabapentin test results are so high that these estimates should not be used alone to estimate patient adherence to dose regimen.

In as much as gabapentin is abused to enhance the experience of other drugs, Figure 1 was assessed for those patients who were positive for gabapentin without a prescription [9,10]. As reported by Smith, et al. gabapentin is often abused in combination with opioids and benzodiazepines [3]. This is confirmed in Figure 1 where prescriptions for benzodiazepines were found in 18% of the patients were positive for gabapentin without a prescription. The rest of the list is an interesting combination of pain medications, stimulants and antipsychotics.

In summary, gabapentin is dosed at high levels, not metabolized and excreted at very high levels in the urine with an equally short half-life. Normalization to creatinine followed by logarithmic transformation can produce a "normal" looking curve, but that curve should not be used to differentiate abuse from adherence without additional studies. A positive gabapentin result in the absence of a prescription is the only clear suggestion of abuse which is apparent at most 34% of the time. Likewise, the absence of gabapentin in urine is the only clear indication the patient is not taking their prescription. While some differences exist between patients prescribed gabapentin and those positive for gabapentin but without an obvious prescription, the high levels of gabapentin in urine prevent a definitive determination of adherence (with prescription) or of abuse (without prescription).

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