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Case Report

A false positive for clozapine using high-pressure liquid chromatography with ultraviolet detection

Lertxundi U, Manrique MH, Echaburu SD, Martinez M. A false positive for clozapine using a high-pressure liquid chromatography with ultraviolet detection method.

Background: We report a case of a false positive for clozapine, when analysing serum levels using a high-pressure liquid chromatography with ultraviolet (HPLC-UV) detection method.
Methods: A patient not taking clozapine tested positive for clozapine three times in two different samples. This false positive was discovered by chance, because of an administrative error made in the first analytic test request.
Results: The analysis of the first sample with a more specific method [HPLC-tandem mass spectrometry (LC-MS/MS)] showed that no clozapine was present.
Conclusions: It is important to acknowledge that depending on the method employed, a false positive should not be ruled out as a possibility. Moreover, and even more worryingly, it should also be taken into account that clozapine serum levels could be tested erroneously high if the unknown interference is present and the HPLC-UV method is used. Although the interfering compound could not be identified, the possibility of a cross-reaction when analysing serum clozapine levels with the HPLC-UV method warrants urgent attention.

Unax Lertxundi[1], Margarita H. Manrique[2], Saioa D. Echaburu[3], Manuel Martinez[4]

[1]Pharmacy Service, Red de Salud Mental de Araba, C/alava 43, 01006 Vitoria-Gasteiz, Araba, Spain; [2]Communitary Rehabilitation Centre, Red de Salud Mental de Araba, C/reyes católicos 6, 01006 Vitoria-Gasteiz, Araba, Spain; [3]Pharmacy Service, Hospital Alto Deba, Avda, Nafarroa 16, 20500 Arrasate Gipuzkoa, Spain; and [4]Reference Laboratory S.A., Barcelona, Spain

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Unax Lertxundi,
Pharmacy Service, Red de Salud Mental de Araba, C/alava 43, 01006 Vitoria-Gasteiz, Araba, Spain.
Tel: +34 945 00 65 33;
Fax: +34 945 00 65 87;
E-mail: Unax.lertxundietxebarria@osakidetza.net

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Clozapine treatment remains the gold standard for treatment-resistant schizophrenia. Therapeutic drug monitoring is usually performed, particularly, in case of partial response or non-response. Serum levels above the therapeutic threshold of 350–420 ng/ml are necessary to determine non-response to clozapine. Depending on the specific method used, reference values may also vary between laboratories (1).

A 58-year-old male diagnosed with schizoaffective disorder was taking the following drugs at the time a routine analytic test was performed:

1 Allopurinol 100 mg: 0-1-0 (Zyloric®, FAES S.A., Madrid, Spain).
2 Biperiden 4 mg: 1-0-0 (Akineton retard®, Desma Lab Farmacéutico, Madrid, Spain).
3 Gemfibrozil 900 mg: 0-0-1 (Lopid®, Pfizer, Alcobendas, Spain)
4 Lithium carbonate 400 mg: 0, 5-0, 5-1 (Plenur®, FAES S.A., Madrid, Spain).
5 Lormetazepam 2 mg at night (Noctamid®, Bayer Hispania, Sant Joan Despi, Spain).
6 Metformin 850 mg: 0-1-0 (Dianben®, Merck S.L, Madrid, Spain).
7 Olanzapine 10 mg: 0-0-1 (Zyprexa velotab®, Lilly, Alcobendas, Spain).
8 Pantoprazol 20 mg: 1-0-0 (Anagastra®, Nycomed Pharma, Madrid, Spain).
9 Extended release quetiapine 300 mg: 1-0-2 (Seroquel prolong®, Astra Zeneca, Madrid, Spain).

The responsible psychiatrist aimed to include a serum lithium level in an analytic request. Because of an administrative mistake, a serum clozapine level was requested instead. The measurement of

the clozapine level was performed by an external reference laboratory in Barcelona.

On the 18th of October 2010, a report of a clozapine level of 472 ng/ml took the responsible psychiatrist by surprise, because of the fact that the patient had no clozapine prescription, and no clozapine serum level was intentionally demanded.

In order to give an explanation to this unexpected finding, the reference laboratory where the analytic test was performed was contacted to check if any administrative error was made in regard to patient/sample identification. But no errors were found whatsoever.

The patient was asked for taking clozapine without prescription, which he denied. No clozapine tablets were found on the patient housing either. Besides, he refuted taking any other drug (different from those listed above), herbal or homeopathic product.

The same sample was re-analysed with the same analytic method, i.e. high-pressure liquid chromatography with ultraviolet (HPLC-UV) detection at 254 nm (2), and once more, it tested positive for clozapine, obtaining an almost identical level (473 ng/ml). But this time, the laboratory staff discovered a slight deviation in the retention time of 10 s with respect to the samples analysed in the same batch. This small deviation had been unnoticed in the first measurement of the sample.

Later, on the 12th of November 2010, the responsible clinician asked for an additional serum clozapine level in a new sample. A clozapine level of 531 ng/ml was reported using the same HPLC-UV method.

Coincidentally, taking advantage of the fact that the laboratory was thinking of changing the method employed to measure clozapine concentrations, among other drugs, the first sample was analysed once again, but with a more specific method, i.e. HPLC-tandem mass spectrometry (LC-MS/MS) instead. No clozapine was found this time.

Suspecting some kind of interference, the reference laboratory analysed some of the drugs the patient was taking and some other antipsychotics with the HPLC-UV method, in order to find out which one was cross-reacting with clozapine. Positive drug samples of olanzapine, quetiapine, metformin and lormetazepam were tested. The available drug positive samples and thus tested antipsychotics were chlorpromazine, levomepromazine, haloperidol and risperidone. All of them, including their respective metabolites gave negative results. Lithium, because of its chemical properties, can be ruled out as a possible interference. This leaves us with gemfibrozil, allopurinol, biperiden or a more complex interference as possible guilty.

Eventually, we interpreted the results as a false positive for clozapine with the HPLC-UV method.

As far as we are concerned, this is the first clozapine false positive reported in the literature. It is important to acknowledge that depending on the method employed, a false positive should not be ruled out as a possibility. Moreover, and even more worryingly, it should also be taken into account that serum clozapine levels could be tested erroneously high if the unknown interference is present and the HPLC-UV method is used.

The patient had a complex treatment comprehending nine different drugs, with their respective metabolites, so sadly it was difficult to glimpse which drug/metabolite or substance was the responsible for the false positive. Nevertheless, this result could have negative effects in the therapeutic relationship between the patient and the clinician. Therefore, although the interfering compound could not be identified, the possibility of a cross-reaction when analysing serum clozapine levels with the HPLC-UV method warrants urgent attention.

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