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Acute eosinophilic pneumonia associated with glyphosate-surfactant exposure

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Acute eosinophilic pneumonia (AEP) is an acute febrile illness that can result in life-threatening respiratory failure. Mechanisms are unclear, but the association with new-onset smoking suggests a possible link (1). Because it may be mistaken for other diseases, especially severe community-acquired pneumonia, the diagnosis may be missed or delayed (2). We report a case of a female patient who developed AEP after recent onset of smoking and extensive unprotected exposure to glyphosate-surfactant. Four years later, during similar circumstances, she was exposed a second time to the same toxic agent, causing a clinical relapse.

A 31-year-old woman was transferred from another hospital with fever (39°C), severe hypoxia and respiratory distress, not responding to antibiotics. A chest radiography showed widespread ill-defined bilateral infiltrates predominantly in the mid- and lower lung zones (Figure 1). The subsequently performed high-resolution computed tomography (HRCT) scan showed areas of ground-glass of a number of peripheral secondary lobules and thickening of interlobular septa. Furthermore, a small amount of bilateral pleural effusion was present. Arterial blood gas analysis on room air revealed a PaO_2 of 6.7 kPa at rest.

The erythrocyte sedimentation rate (ESR) 53 mm/hr, C-reactive protein (CRP) 230 mg/l, lactate dehydrogenase (LDH) 549 U/l (<250U/L) were increased. Total white blood cell (WBC) count $13.5x10^9$/l and eosinophils 1.0% were within normal ranges.

Bronchoalveolar lavage (BAL) fluid cell differentiation analysis showed an increased level of eosinophils (45% of the total cell count; see Figure 2 and Table 1). No bacteria were seen and the culture of BAL fluid remained sterile.

An thorough exposure history revealed that a few days before the onset of symptoms the patient had helped for at least six hours to clean up the school yard of her daughter using large amount of herbicides containing glyphosate-surfactant (Roundup, probably 41% glyphosate isopropylamine + 15.4% polyoxyethyleneamine (POEA)). It happened to be a nice warm day, she used the herbicide without any protection. Moreover, she restarted smoking three weeks before this first episode of exposure to glyphosate-surfactant. According to the BAL fluid analysis the diagnosis AEP was confirmed and treatment with corticosteroids was started. The clinical condition of the patient improved within a few days. Dyspnea and cough disappeared. After recovery the chest radiograph abnormalities cleared (Figure 1b). The PaO_2 returned within normal limits (11.8 kPa on room air at rest).

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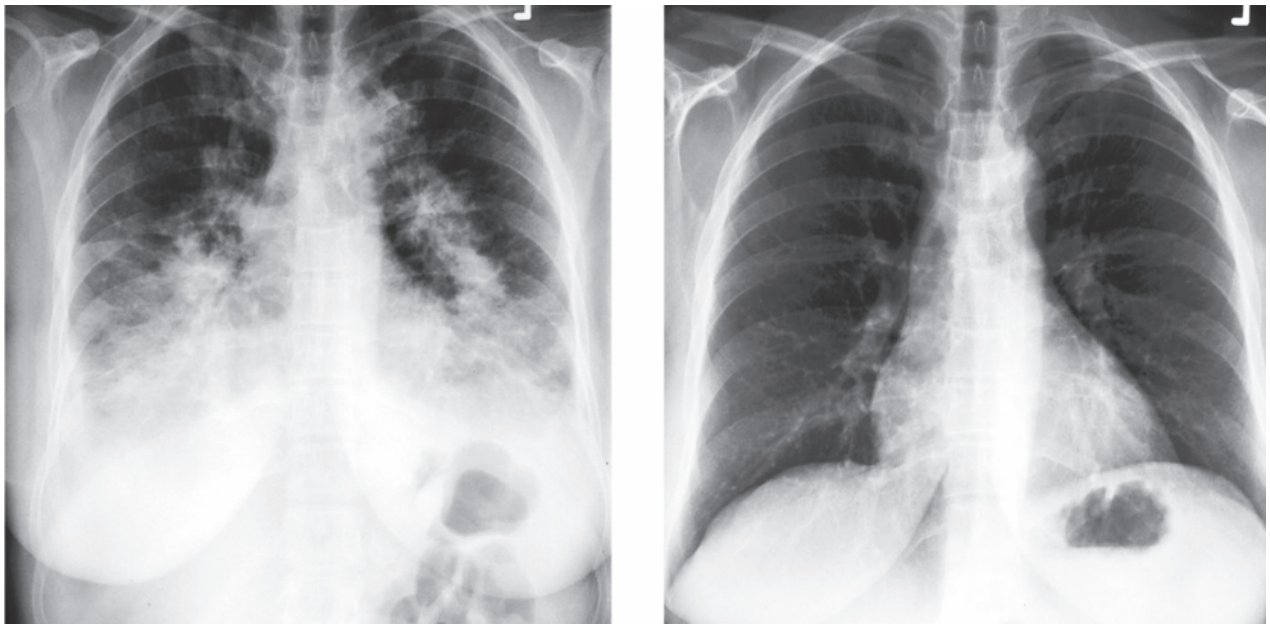

Fig. 1. Chest radiography showing widespread ill-defined bilateral infiltrates predominantly in the mid- and lower lung zones (left); complete clearance of the abnormities on the left after 2 weeks treatment with glucocorticoids

Four years later the patient visited the emergency room with a non-productive cough, dyspnea, and fever (38.5°C). Her chest radiograph showed bilateral infiltrates. She restarted smoking again two weeks prior and smoked 15 cigarettes the day before. Three days before the onset of symptoms there was a second episode of significant unprotected exposure to glyphosate. She confirmed that she did not had any contact with glyphosate in the period after the first episode until three days before. To date, she restarted smoking again in the last four years at least three times.

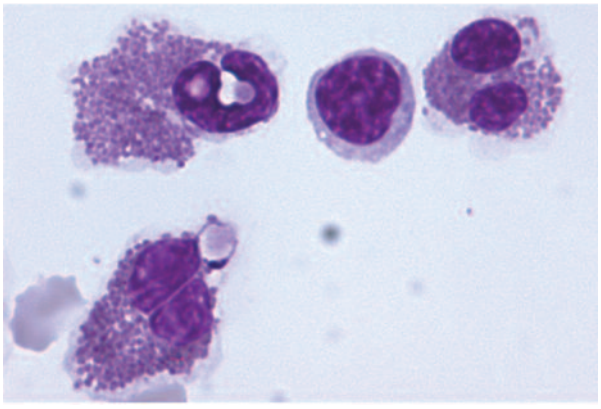

Fig. 2. May-Grünwald Giemsa stain of the bronchoalveolar lavage fluid (BALF) obtained from the presented case with acute eosinophilic pneumonia (AEP). This figure shows three eosinophils surrounding a lymphocyte. Eosinophils have nuclei which often consist of two lobes. Their cytoplasma contains large bright orange colored granules. Magnification: 1000x

At hospitalization laboratory findings were increased for CRP (140 mg/l), eosinophils ($700x10^6$/l) and LDH (345 U/l). ESR (28 mm/hr) and total WBC ($7.8x10^9$/l) were within normal ranges. Arterial blood gas analysis on room air showed a PaO_2 of 9.5 kPa at rest.

Results from chest radiography, HRCT and BAL fluid analysis were comparable with results from the first episode (see also Table1).

Additionally, cytochrome P450 (CYP) genotyping revealed a heterozygous *CYP2C19* (*1/*2) and no polymorphisms in the *CYP2D6* and *CYP2C9* genes. The *1 allele of the *CYP2C19* gene codes for a fully functional enzyme, but the *2 produces an enzyme with decreased function resulting in an overall lower enzymatic activity or a so-called intermediate metabolizer (3).

Glyphosate-based herbicides are worldwide and extensively used to eliminate weeds. Most glyphosate-based products contain a surfactant which helps glyphosate to penetrate plant cells. In

Table 1. Results of the bronchoalveolar lavage (BAL) fluid analysis of the reported patient with two episodes of acute eosinophilic pneumonia (AEP). The relapse after re-challenge (episode 2) occurred 4 years after the first episode of AEP

	Episode 1	Episode 2
Total cell count	$40.0x10^4$/ml	$76.8x10^4$/ml
Alveolar macrophages	42.1%	10.2%
Lymphocytes	7.6%	17.2%
Neutrophils	6.2%	0.2%
Eosinophils	45.0%	67.8%
Mast cells	1.0%	4.6%
Culture	negative	negative
Fe-staining	negative	negative

vitro studies evaluating glyphosate in human primary cells and cell lines show that the cytotoxicity of commercial formulations of glyphosate, containing the surfactant POEA, is much higher than the active component itself (4-7). This might lead to an underestimation of the toxicity of glyphosate-based herbicides compared to the active component glyphosate only. Acute poisoning with a glyphosate-surfactant herbicide after oral ingestion can cause gastrointestinal irritation, hepatic and renal dysfunction, cardiovascular instability, aseptic meningitis, and pulmonary insufficiency. Respiratory distress is a factor of poor outcome and mortality after ingestion of glyphosate-surfactant (8-10). In a retrospective analysis of 601 cases with acute poison exposure due to ingestion of glyphosate containing herbicides, the majority (64%) developed signs of only minor poisoning. Aspiration pneumonitis was reported in 8% of the patients in this group (8). A case of acute intoxication after exposure for a considerable period of time to solvent vapors containing a glyphosate-surfactant herbicide resulted in toxic pneumonitis (11).

Many pesticides are subjected to metabolic biotransformation by CYP enzymes. Sub-lethal exposure of rats to glyphosate-surfactant in drinking water showed enhanced glutathione transferase enzyme activities (phase II reactions), next to levels of reduced glutathione and lipid peroxidation in the liver, kidneys and small intestine (12). This implies involvement of the CYP system (phase I reactions) in the detoxification of glyphosate-surfactant. Important CYP enzymes involved in the metabolism of common prescription drugs and other xenobiotics are CYP2D6, CYP2C9, and CYP2C19 (13). The genes coding for these enzymes are highly polymorphic and allelic variants code for enzymes that have reduced or no metabolic activity.

In a study where pesticide inhibition of all major xenobiotic-metabolizing enzymes was measured in human hepatic microsomes, glyphosate was shown to be a potent inhibitor of CYP2C9 (and CYP2C8) but it did not inhibit CYP2C19 and CYP2D6 (14). Since the mechanisms of chemicals like pesticides are comparable to drugs, drug-induced ILD could be expanded to xenobiotic-induced ILD (13).

Therefore, in future studies next to 'personalized medicine' also 'personalized toxicity' should be taken into account for diagnoses and treatment.

The presented case developed AEP after recent onset of smoking and extensive exposure to glyphosate-surfactant. The additional exposure associated with the recent start of smoking may have contributed to the development and/or severity of AEP (15). Relapse after re-challenge both with smoking and glyphosate-surfactant made the association highly likely. The absence of BAL analysis results in glyphosate-based poisoning reports may explain that AEP related to glyphosate-surfactant exposure was not considered before. A similar case of AEP was described after phosgene inhalation after 4 weeks of smoking (16). In this case a smoking provocation test, without phosgene, did not lead to recurrence of AEP symptoms assuming that the additional chemical agent was responsible for the development of smoking-related AEP in the described case. Moreover, the results of a study among 183,000 military personnel where 18 cases of AEP were reported that were linked with new-onset of smoking and significant exposure to fine airborne sand or dust contributes to this hypothesis (17). Additionally, we found that the patient was heterozygous for *CYP2C19* (*1/*2). It is tempting to speculate that next to the inhibition of CYP2C9, her genotype causing a - at least partly - changes in the metabolization of glyphosate and in combination with smoking derived compounds may have contributed to the development and/or severity of AEP. In line with Tamada et al. (16) we suggest that not only cigarette smoking but also the inhalation of an additional irritant - such as the toxic gas glyphosate - might be necessary for the development of cigarette smoking-related AEP. This is supported by the

fact that in this case only restarting smoking did not cause an episode of AEP. This case highlights the importance of a thorough exposure history e.g., possible occupational and environmental exposures together with drug-intake. Genotyping should be considered in cases of severe unexplained pulmonary damage. It also warrants further research to investigate to which degree toxicity of glyphosate-surfactant might be associated with reduced metabolic capacity of CYP enzymes.

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