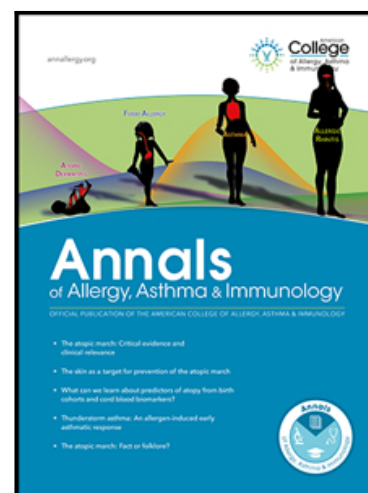


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Suran L Fernando MB BS BSc (Med) PhD FRACP FRCPA ,
Jamma Li MB BS MPhil FRACP FRCPA ,
Chris Toon MB BS FRCPA , Chris Weir BSc PhD

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Acute generalized exanthematous pustulosis to a novel oral anticoagulant (apixaban)

Suran L Fernando^{1,2,3} MB BS BSc (Med) PhD FRACP FRCPA, Jamma Li^{1,2,3} MB BS MPhil
FRACP FRCPA, Chris Toon MB BS FRCPA⁴, Chris Weir BSc PhD^{2,4}

¹Department of Clinical Immunology and Allergy, Royal North Shore, Sydney, Australia

²Immunology Laboratory, New South Wales Health Pathology, Royal North Shore, Sydney,
Australia

³University of Sydney, Sydney, Australia

⁴Department of Anatomical Pathology, New South Wales Health Pathology, Royal North
Shore, Sydney, Australia

Corresponding author: Suran L Fernando

Address: Department of Clinical Immunology and Allergy
Reserve Road
St Leonards
NSW 2065
Australia

Phone: +61299264108

Fax: +61294631058

Email: suran.fernando@health.nsw.gov.au

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Non-vitamin K antagonist oral anticoagulants or novel oral anticoagulants (NOACs) are increasingly used in the prevention of stroke in atrial fibrillation and in the prevention and treatment of venous thromboembolism (VTE). NOACs include direct thrombin inhibitor dabigatran and factor Xa inhibitors apixaban, edoxaban and rivaroxaban. Severe cutaneous adverse reactions are rare with definite cases of drug reaction with eosinophilia and systemic symptoms (DRESS) attributed to both rivaroxaban and apixaban^{1,2}. No published cases to date have been described of acute generalised exanthematous pustulosis (AGEP) to a NOAC. Mahmoud et al, used the FDA Adverse Event Reporting System and the Sentinel System to suggest a causal relationship between NOACs and cutaneous small vessel vasculitis³. A case series of delayed hypersensitivity reactions to apixaban featured 2 patients with IgA cutaneous vasculitis, one with cutaneous palpable purpura, and another with drug-induced SLE⁴. We present a case of AGEP to apixaban including the findings of cellular in vitro studies involving basophil activation and lymphocyte transformation.

A 54-year-old female developed multiple large bilateral pulmonary emboli on a background of previous venous thromboembolism, an inferior vena cava filter, and heparin-induced thrombocytopenia and thrombosis syndrome. She was commenced on apixaban 10 mg every 12h and 10h later developed a pruritic rash with pinpoint pustules over her nuchal folds and a neutrophilia ($13.9 \times 10^9/L$). A biopsy of an axillary lesion was consistent with early AGEP with neutrophilic dermatosis and intraepidermal pustulosis and absence of epidermal necrosis. Her only intercurrent medication was sertraline commenced for depression 6 years previously. A diagnosis of apixaban-DRESS was made and her rash resolved 17 days later with cessation of the drug, and the application of topical betamethasone dipropionate 0.05% every 12h for 3 weeks. The rash did not flare with the commencement of warfarin with danaparoid-bridging.

A basophil activation test (BAT) was performed 6 weeks following the resolution of the rash on serial dilutions of apixaban (1 $\mu g/mL$ to 400 $\mu g/mL$) as per our previously published method⁵. There was a 52% activation of the patient basophils at a concentration of 400 $\mu g/mL$ of apixaban in comparison to 20% activation of basophils for the positive control and no activation of basophils for an age and gender matched negative control.

Fresh heparinized blood was collected to run the lymphocyte transformation test (LTT) 3 months following the resolution of the rash. Blood was layered on Histopaque®-1077 (Sigma-Aldrich, UK) at a ratio of 1:1 and centrifuged for 20 mins at 1200 rpm with no brake. PBMCs were collected at the interface and washed in sterile PBS and spun at 1400 rpm for 7 mins to collect PBMCs. Cells were then counted and plated out at 200,000 cells per well in RPMI with 10% FCS and penicillin-streptomycin (Sigma-Aldrich, UK) on a 96 well plate. Apixaban was solubilized in sterile water at 100 $\mu g/mL$ stock solution. Apixaban was added onto PBMCs at 100, 50, 25, 12.5, 6.2 and 3.1 $\mu g/mL$ in triplicate. PHA at 5 $\mu g/mL$ (Sigma-

Aldrich, UK) and T-Cell Transact™ beads (Miltenyi Biotec, Germany) at a 1:200 dilution were used as positive controls. Negative control wells contained only RPMI media. The plate was incubated at 37°C for 6 days to allow for cell-drug interaction. At day 6, 50µl XTT (Roche, Germany) was added to all wells to measure cellular metabolic activity as an indicator of cell viability, proliferation and cytotoxicity. In the presence of the electron-coupling reagent, XTT is reduced and converted to an orange-colored formazan product, which is read at 4, 8 and 24hrs at an absorbance of 492 and 690nm. Straight RPMI with XTT added was used as control blanks. There was a significant T cell response to apixaban at concentrations of 6.2µg/ml to 25µg/ml for the patient as compared to the negative control (Figure1).

This is the first definite reported case of AGEF to a NOAC confirmed by histopathologic findings and supported by in vitro studies. Patch testing confirmation for AGEF usually shows poor sensitivity. A study by Pinho et al, showed a patch test reactivity of 18.1% for AGEF to antibiotics⁶. Positive LTTs for the suspected drugs are observed in greater than 50% of analyzed patients who experience certain drug-induced skin rashes such as maculopapular rash, AGEF, DRESS as compared to less than 10% of patients for Stevens-Johnson syndrome/Toxic Epidermal Necrolysis, vasculitis, or non-cutaneous manifestations of ADR⁷. In contrast the specificity is very high especially for antibiotics (β-lactam antibiotics and sulphonamides) and anticonvulsant agents (carbamazepine and lamotrigine)⁸. The most intriguing aspect of the case is the positive BAT result. The assay is performed to determine the IgE-mediated reaction but its utility and performance characteristics in AGEF, a type IV sensitivity of early onset, often within hours of exposure of the drug is unknown. The pathogenesis of AGEF is still to be fully elucidated and involves drug-specific CD4+ or CD8+T cells producing copious amounts of CXCL8 and GM-CSF resulting in reduced neutrophil apoptosis and accumulation of neutrophils within the skin⁹. Mast cell degranulation in AGEF has not been studied extensively to date. It has been reported in

AGEP-like cases with suspected drugs (ampicillin, cefazolin, tetracycline, griseofulvin, enalapril maleate) although the non-IgE mediated pathway involved has not been determined¹⁰.

In conclusion, we describe the first case of NOAC-AGEP that is confirmed with LTT and has the intriguing finding of a positive BAT raising further speculation on the role of mast cell degranulation in the pathogenesis of AGEp. Further studies of the utility of BAT and LTT may be warranted in AGEp to determine for the possibility of mixed reactions or culprit drug-specific mechanisms.

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References

1. Radu C, Barnig C, de Blay F. Rivaroxaban-Induced Drug Reaction With Eosinophilia and Systemic Symptoms. *J Investig Allergol Clin Immunol* 2016; 26(2):124-6.
2. Menteşoğlu D, Doğan Günaydın S, Ersoy-Evans S. Drug reaction with eosinophilia and systemic symptoms syndrome induced by apixaban. *Dermatol Ther*. 2020;33:e13719.
3. Mohamoud M, Horgan C, Eworuke E, Dee E, Bohn J, Shapira O, et al. Complementary Use of U.S. FDA's Adverse Event Reporting System and Sentinel System to Characterize Direct Oral Anticoagulants-Associated Cutaneous Small Vessel Vasculitis. *Pharmacotherapy*. 2020;40:1099-1107.
4. Isaq NA, Vinson WM, Rahnama-Moghadam S. Apixaban-induced cutaneous hypersensitivity: a case series with evidence of cross-reactivity. *Dermatol Online J*. 2020;26:13030/qt1r37k272.

5. Li J, Best OG, Mulligan SP, Fernando SL. Serum from a subset of patients with chronic lymphocytic leukemia and large local reactions to mosquito bites induces upregulation of CD63 surface expression on basophils in atopic donors. *Leuk Lymphoma*. 2016;57:2417-20.
6. Pinho A, Coutinho I, Gameiro A, Gouveia M, Gonçalo M. Patch testing - a valuable tool for investigating non-immediate cutaneous adverse drug reactions to antibiotics. *J Eur Acad Dermatol Venereol*. 2017;31:280-287.
7. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. *Allergy*. 2004;59:809–20.
8. Möbs C, Pfützner W. Cellular in vitro diagnosis of adverse drug reactions. *Allergo J Int*. 2014;23(5):164-171.
9. Fernando SL. Acute generalised exanthematous pustulosis. *Australas J Dermatol*. 2012;53:87-92
10. Lazarov A, Livni E, Halevy S. Generalized pustular drug eruptions: confirmation by in vitro tests. *J Eur Acad Dermatol Venereol*. 1998;10:36-41.

Figure Legend

Lymphocyte transformation test results show significant memory T cell response to apixaban at 6.2-25 μ g/ml concentrations (** $p < 0.005$ by one way ANOVA). The dotted line represents the negative control cut off. Both positive controls were also significantly higher than negative control (** $p < 0.005$).

