

**P002** Early membrane events in neutrophil (PMN) apoptosis:  
Membrane blebbing and vesicles release, CD43 and CD16  
down-regulation and phosphatidyl serine externalization.  
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CD43 down-regulation during PMN apoptosis is not caused by proteolysis or internalization. Could it be released with blebs-derived membrane vesicles? Membrane blebbing was followed (optic, fluorescence, time lapse microscopy) on PMN "synchronised" by an overnight incubation at 15°C before their spontaneous apoptosis at 37°C. Released vesicles were analysed and quantitated by flow cytometry. Membrane blebbing, release of blebs-derived membrane vesicles, decrease of CD43/CD16 expression and phosphatidylserine externalization occurred simultaneously. However, caspase (zVad-FMK) and PKC (staurosporin, bisindolyl maleimide) inhibition prevented annexin binding but neither blebbing, vesicles release nor CD43 expression decrease; MLCK inhibitor ML9 prevented cell blebbing and vesicles release but had no effect on CD43/CD16 down-regulation or annexinV binding. By electron microscopy, CD43 appeared poorly expressed on membrane blebs and concentrated at bleb "necks", thus possibly involved in cytoskeleton-membrane anchorage at actomyosin contraction sites. We here show that cell blebbing, phospholipids "flip-flop" and CD43/CD16 down-regulation are simultaneous membrane events that occur independently from one another.