Circulating miRNAs – a potential tool to identify severe asthma risk?

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Although one of the commonest chronic diseases, asthma lacks a gold standard diagnostic test. Reliance on a clinical diagnosis alongside the heterogeneous nature of asthma means that asthma diagnosis is often delayed to the detriment of the patient. Therefore, new asthma diagnostic biomarkers are an important research focus. MicroRNAs (miRNAs) might hold promise in that regard. These small noncoding RNAs act through RNA-induced silencing complexes to post-transcriptionally regulate mRNA (messenger RNA). A previous study demonstrated differential miRNA expression in blood eosinophils from asthmatics compared with healthy individuals. They clustered asthmatics and healthy subjects showing that miRNA profiles in eosinophils (especially miR-185-5p and miR-1246) are potential serum biomarkers for ranking asthma severity.(1) Another study from our lab demonstrated that miR-150, miR-152, and miR-375 are up-regulated in severe asthma.(2)

We determined the differential miRNA expression in sera of severe asthmatic patients and compared it to mild asthma and no asthma to assess their potential as biomarkers for the diagnosis of asthma and its discriminative capability for asthma severity.

We investigated the presence of 175 miRNAs in serum samples collected from 12 adult subjects (4 healthy, 4 mild asthma, and 4 severe asthma) of our cohort studies (summary features and definitions, Table 1). Healthy subjects and mild asthma patients were from the Isle of Wight Birth Cohort (IOWBC) (3), while severe asthma patients were from the Wessex AsThma CoHort of difficult asthma (WATCH) study (4). We hypothesized that asthma-specific miRNAs may differentiate asthmatic from healthy subjects, while other miRNAs may differentiate mild from severe asthma.

We extracted miRNAs from stored serum using miRNeasy Serum/Plasma Kit (QIAGEN). After extraction, RNA was reverse-transcribed to cDNA. We then evaluated miRNA expression by quantitative PCR using Human Serum/Plasma Focus, miRCURY LNA miRNA Focus PCR Panel kit (QIAGEN) which is designed for profiling 175 human miRNAs commonly found in serum and plasma. Results were analysed using QIAGEN

web portal GeneGlobe. At least two-fold change in expression and P [?]0.05 were required to consider the miRNA of diagnostic value.

Figure 1 shows volcano plots for comparisons of the studied miRNAs in severe asthmatic, mild asthmatic, and healthy groups. We found that, miR-193a-5p was significantly increased while miR-181a-5p, miR-146a-5p, and miR-16-2-3p were significantly decreased in serum from severe asthmatics compared to healthy subjects. Furthermore, miR-197-3p, miR-223-3p, miR-151a-3p, miR-191-5p, and miR-28-3p were significantly increased and miR-451a, miR-16-2-3p, miR-210-3p, miR-133a-3p, miR-660-5p and miR-144-3p significantly decreased in serum from severe asthmatics compared to mild asthmatics. There were no significant differences in expression of the studied miRNAs in serum between healthy subjects and mild asthma patients.

Our preliminary study demonstrated differential sera miRNA expression in severe asthma compared to both mild asthma and healthy subjects. We also found that different miRNAs respectively distinguished severe asthma from mild asthma than from healthy controls. Such findings emphasise the distinctive nature of severe asthma and may aid understanding of mechanistic differences between mild and severe asthma. To our knowledge, miR-28-3p, miR-16-2-3p, and miR-210-3p have not been previously reported as differentially expressed miRNAs in asthma while miR-151a-3p has been inversely correlated with Bronchoalveolar lavage (BAL) eosinophil percentage in asthma patients.(5) We tested 175 miRNAs within a standard panel but other miRNAs previously reported in the literature may differentiate asthmatic patients from healthy subjects.(1, 2) Furthermore, asthma is a heterogeneous condition so different miRNAs may be relevant to different phenotypes and endotypes. Differential miRNA expression may be seen in different tissues too. Our pilot study shows that miRNA expression may help to distinguish severe asthma status. Larger scale work studying miRNA expression in different asthma subgroups, different tissue compartments and using a wider array miRNAs is therefore warranted.

Table1: Clinical characteristics for subjects from different groups.

	Severe asthma N=4	Mild asthma N=4	Healthy N=4
Definition of Asthma Status	Enrolled in WATCH study and taking BTS steps additional controller therapies/specialist therapies (6)	Enrolled in IOWBC. Physician diagnosis of asthma at age 18-years plus either wheeze within the last 12 months or currently taking asthma medication at BTS steps regular preventer/ initial add on therapy (6)	Enrolled in IOWBC. No documented history of asthma ever at age 18-years
Gender (F) % (N)	75 (3)	75 (3)	100 (4)
Current Smoker % (N)	0 (0)	50 (2)	25 (1)
Never smoked % (N)	50 (2)	25 (1)	75 (3)
ICS dose BDP equivalent µg/day	2500 (1000-3000)	400 (0-800)	NA
BMI (Kg/m^2)*	31.8 (22.1-39.6)	23.9 (20.2-26.4)	21.7 (19.0-24.6)
Rescue OCS (in last 12 months) (N)*	3.7 (1-7)	0	0
On Maintenance OCS	No	No	No
On Asthma Biologics % (N)	0 (0)	0 (0)	0 (0)
FEV1 (%)*	80.84 (68.73-96.76)	3.88 (2.97-5.37)	3.69 (3.54-4.03)
FVC (%)*	94.63 (82.88-106.72)	4.68 (3.34-6.96)	4.15 (3.83-4.45)
Total IgE (KU/L)*	1463.7 (19.6-2907.9)	119.4 (5.6-346.0)	210 (36-384)

	Severe asthma N=4	Mild asthma N=4	Healthy N=4
Atopy % (N)	50 (2)	75 (3)	50 (2)

Data are presented as %, mean. BMI: body mass index; ICS: inhaled corticosteroids; BDP: beclometasone dipropionate; OCS: oral corticosteroids; FEV1: forced expiratory volume in 1 s; FVC: Forced vital capacity. *Mean (range). NA = Not applicable WATCH= Wessex AsThma CoHort. IOWBC= Isle of Wight Birth Cohort. BTS=British Thoracic Society.

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